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Anhydrol Forte

20% w/v aluminium chloride hexahydrate

The effective new roll-on solution for hyperhidrosis

Developed to utilise the proven effectiveness of 20% w/v aluminium chloride hexahydrate whilst minimising the possibility of irritation and soreness, Anhydrol Forte is a new proprietary solution presented in a specially designed roll-on bottle.

The consistent formulation of Anhydrol Forte saves the pharmacy valuable time and ensures that patients receive an identical preparation wherever their prescriptions are dispensed.

Anhydrol Forte is *not* a deodorant but an antiperspirant that works by inhibiting the activity of the sweat glands even below the level of the stratum corneum.

For effective control of hyperhidrosis — new Anhydrol Forte, the roll-on solution in a controlled delivery system.

Full prescribing information, including data sheet is available from:
Dermal Laboratories Limited, Gosmore, Hitchin, Herts. SG4 7QR

Prescribing Information

PRESENTATION:
Colourless evaporative solution containing 20% w/v aluminium chloride hexahydrate.

INDICATION:
For the topical treatment of hyperhidrosis specifically involving the axillae, hands or feet.

DIRECTIONS FOR USE:
Apply to the affected sites at night as required and allow to dry. Wash off in the morning.

PRECAUTIONS:
Restrict application to affected sites only.
Keep away from the eyes.

PACKAGE QUANTITY AND BASIC NHS PRICE:
10ml glass roll-on bottle £2.25.

REFERENCE:
British Medical Journal, 1978, **2**, 84.

PL 0173/0030

Anhydrol is a registered trade mark

Squ[illegible] out [illegible] gel

rub in a lotion.

Although presented in a tube, 'Synalar' Gel behaves, upon application, as an aqueous solution.

Containing a minimum of gelling agent, it becomes, under friction, a watery, greaseless liquid which rubs into the scalp easily and completely.

Yet, 'Synalar' Gel is more controllable than a true solution and therefore less wasteful; it is non-irritant and cools without excessive drying.

With all the advantages of a solution, and few of the disadvantages, 'Synalar' Gel provides a unique answer to the problems of scalp dermatoses.

Synalar gel
Fluocinolone acetonide
The solution in a tube.

Prescribing Notes

Presentation 'Synalar' Gel consists of Fluocinolone Acetonide BP 0.025% w/w, presented as a clear, water-miscible gel. **Uses** Specific indications for 'Synalar' Gel are seborrhoea, seborrhoeic dermatitis and psoriasis of the scalp. **Dosage and administration** A small quantity of 'Synalar' Gel is massaged into the scalp night and morning using the finger tips. **Contra-indications, warnings, etc.** 'Synalar' Gel is contraindicated in tuberculous, fungal, syphilitic and most viral lesions of the skin, and acne rosacea. Where there is a bacterial infection associated with an inflammatory skin condition, 'Synalar' should only be administered if adequate antibacterial cover is also given. Topical administration of corticosteroids to pregnant animals can cause abnormalities of fetal development. Although the relevance of this finding to human application has not been established, when topical steroid treatment is considered necessary during pregnancy both the amount applied and the length of treatment should be minimised. Long term continuous topical steroid therapy should be avoided since adrenal suppression can occur, particularly when infants are being treated. With 'Synalar' Gel side effects are extremely rare, but, as with all topical corticosteroids, the occasional patient may show an adverse reaction. **Product licence number** 0029/5039 **Basic NHS Price:** £1.15 (30g tube). 'Synalar' is a trade mark. Full prescribing information on all 'Synalar' preparations is available upon request.

ICI

Imperial Chemical Industries Limited,
Pharmaceuticals Division,
Alderley House, Alderley Park,
Macclesfield, Cheshire.

Synalar gel

Fluocinolone acetonide

The solution in a tube.

flamazine
®
Silver Sulphadiazine 1% w/w Topical antibacterial cream
FLAMAZINE PRESENTS THE BROAD SPECTRUM ANTIBACTERIAL
ACTION OF SILVER IN A NON STAINING AND SOOTHING CREAM

Further information is available on request from:

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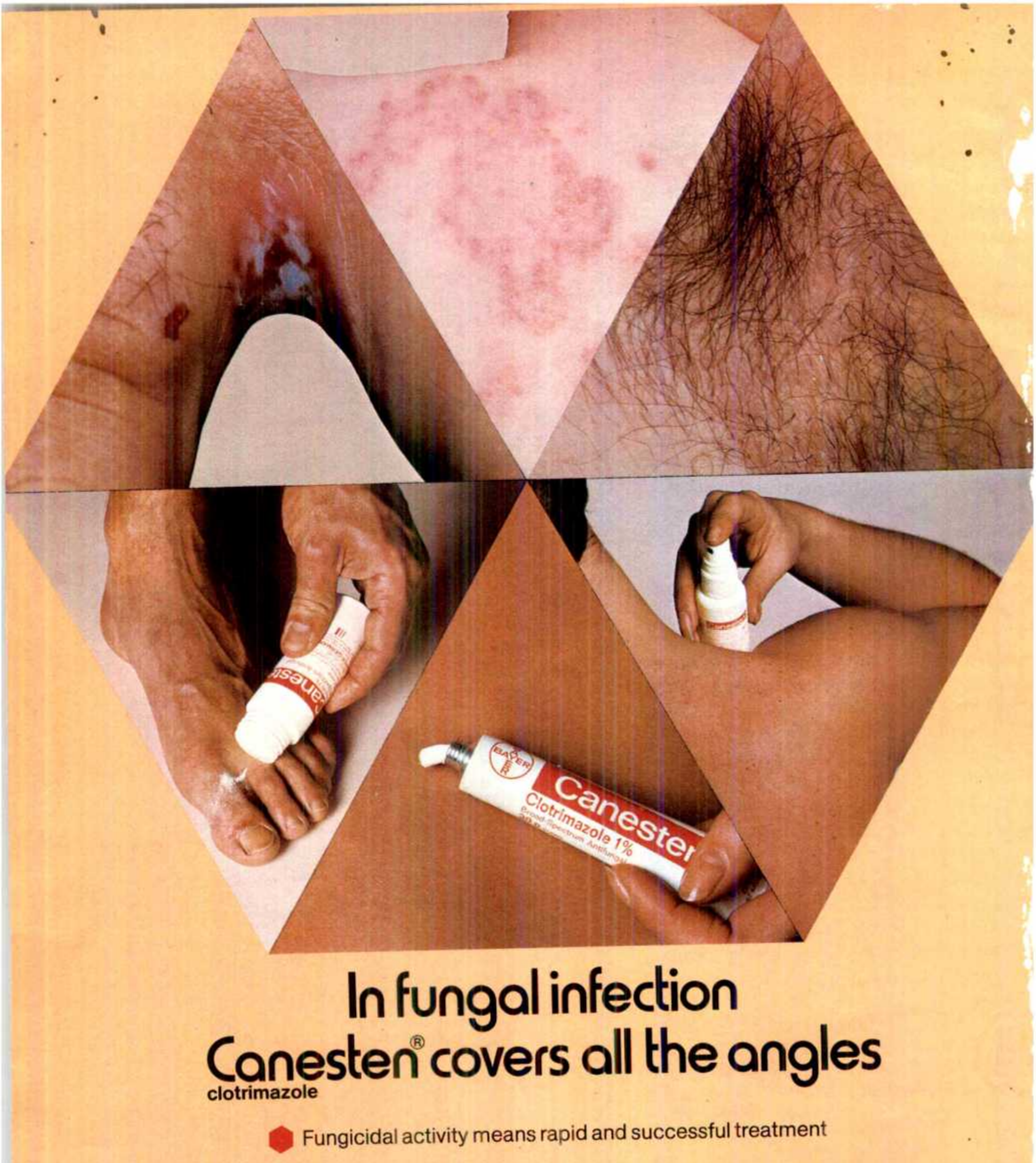


In fungal infection Canesten® covers all the angles

clotrimazole

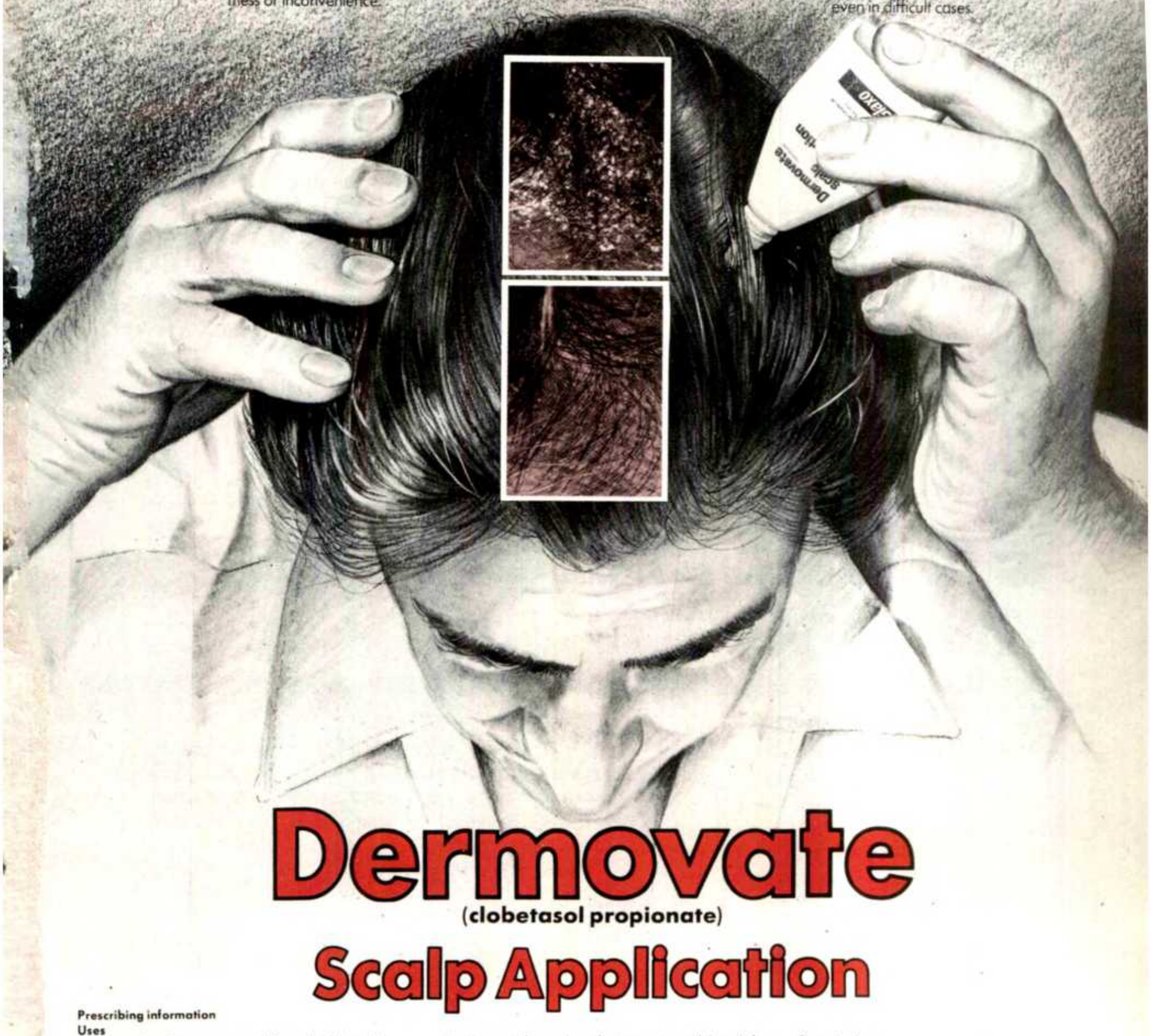
- Fungicidal activity means rapid and successful treatment
- Free from grease, stain and smell
- A range of presentations for all body areas
- Cost-effective routine antifungal therapy

Prescribing information: Presentations *Cream* A white cream containing 1.0% clotrimazole. *Atomiser Spray* A clear solution containing 1.0% clotrimazole in 30% isopropanol. *Powder* A wh
containing 1.0% clotrimazole. **Indications** All fungal skin infections due to dermatophytes, yeasts, moulds and other fungi including ringworm (tinea) infections. **Dosage and Administrati**
Atomiser Spray Apply to the affected area 2-3 times daily. *Powder* as above and may also be dusted inside articles of clothing and footwear which are in contact with the infected area as a pr
against re-infection. **Side-effects** Rarely, patients may briefly experience a transient local mild burning or irritation. **Precautions** Canesten atomiser spray should not be used near a
naked flame, should not be allowed to come into contact with the eyes, ears or mucous membranes and should not be inhaled. **Product Licence Numbers** Cream: PL 0010/0016
Atomiser Spray: PL 0010/0060 Powder: PL 0010/0067 **Basic NHS Prices** Cream: 20g, £1.52 50g, £3.55 Atomiser Spray: 40ml, £4.08 Powder: 30g, £1.10. Further information
is available from Bayer UK Limited, Pharmaceutical Division, Haywards Heath, West Sussex RH16 1TP. Telephone: Haywards Heath (0444) 57911. Distributed in the Republic of Ireland by
Bayer (Ireland) Limited, Dun Laoghaire, County Dublin. ®Registered Trade Mark of Bayer, Germany.

Our Application is often successful, even in difficult cases

Dermovate Scalp Application has been specially developed for difficult, stubborn scalp dermatoses. The preparation, contained in a plastic squeeze bottle with an elongated nozzle, is easily applied through the hair direct to the scalp where it spreads evenly over the lesions – with no mess or inconvenience.

Dermovate Scalp Application is odourless and leaves no visible residue on the hair. The alcohol/water base dries quickly producing an immediate cooling effect accompanied by relief from itching and inflammation. Twice daily application is often successful in rapidly clearing the lesions, even in difficult cases.



Dermovate

(clobetasol propionate)

Scalp Application

Prescribing information

Uses

Psoriasis and recalcitrant eczemas of the scalp. Clobetasol propionate is a highly-active topical corticosteroid which is indicated for use in short courses for conditions which do not respond satisfactorily to less active steroids.

Dosage and administration

Apply sparingly to the scalp night and morning until improvement occurs. As with other highly-active topical steroid preparations, therapy should be discontinued when control is achieved. Repeated short courses of Dermovate Scalp Application may be used to control exacerbations. If continuous steroid treatment is necessary, a less potent preparation should be used.

Contra-indications

Infections of the scalp. Hypersensitivity to the preparation.

Precautions

Care must be taken to keep the preparation away from the eyes. Do not use near a naked flame. Long-term continuous therapy with Dermovate Scalp Application should be avoided, particularly in infants and children, in whom adrenal suppression occurs readily.

Development of secondary infection requires withdrawal of topical corticosteroid therapy and institution of appropriate systemic antimicrobial therapy.

Topical administration of corticosteroids to pregnant animals can cause abnormalities of fetal development.

In general, topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

Dermovate preparations are usually well tolerated, but if signs of hypersensitivity appear, application should be stopped immediately. As with other topical corticosteroids, local atrophy may result from prolonged treatment, particularly if occlusive dressings are used. When extensive areas are treated, sufficient systemic absorption may occur to produce the features of hypercorticism, especially in infants and children. This effect is more likely to occur after prolonged treatment, particularly if occlusive dressings are used. In rare instances, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Presentation

Plastic squeeze bottle with elongated nozzle containing 25 or 100ml

Basic NHS cost (exclusive of VAT)

25ml bottle £2·72

100ml bottle £9·65

Product Licence number

4/0242

Glaxo

Leaders in topical steroid therapy

Further information on Dermovate (trade mark) Scalp Application is available from:

Glaxo [illegible]

THE UNIVERSITY OF LIVERPOOL

Mersey Regional Council for Postgraduate Medical Education

2-Day Course In Paediatric Dermatology

Thursday 30th April and Friday 1st May 1981

A course for Senior Registrars in Dermatology, limited to 12 participants. Application has been made for approval under the Advanced Postgraduate Training Scheme.

Full details from: Dr. J.L. Verbov/Dr. C.F.H. Vickers,
c/o Postgraduate Medical Dean's Office,
Duncan Building,
University of Liverpool,
PO Box 147,
LIVERPOOL L69 3BX. Tel: 051-709 3114

Dermatological Nursing and Therapy

R.H. Seville and E. Martin, both of Beaumont Hospital, Lancaster

This is a concise nursing text which classifies skin disorders and details the nursing therapy required. Common conditions and rare complaints are introduced and described and the book emphasises the importance of nursing management. The text is highly readable; it is enhanced by numerous black and white and colour illustrations and forms a useful manual for those concerned with the care and treatment of patients suffering from skin diseases.

1981. 240 pages, 113 illustrations.
Paper, £7.50

Contents:
Dermatological nursing; Psychosomatic dermatology; Psoriasis, introduction to dressings; Eczema, infantile eczema; Prurigo, neurotic excoriations, artefacts, trichotillomania, alopecia areata, lichen planus; Seborrhoeic dermatitis, acne, rosacea, anogenital pruritus; Urticaria and drug eruptions; Dermatitis; Immunology, blistering diseases; Cutaneous manifestations of systemic disease and malignancy; Granulomata including syphilis; Infections—bacterial, fungal and viral; Infestations; Leg ulcers; Some problems in the elderly; Tumours—benign and malignant; Procedures—biopsy, therapeutic and cosmetic; Investigations—histological, mycological and bacteriological; PUVA, X- and Grenz-ray therapy, physiotherapy, chiropody, dietetics; The dermatology team; Structure, function and care of the skin, nails and hair; Principles of physical treatment and preparations to avoid.

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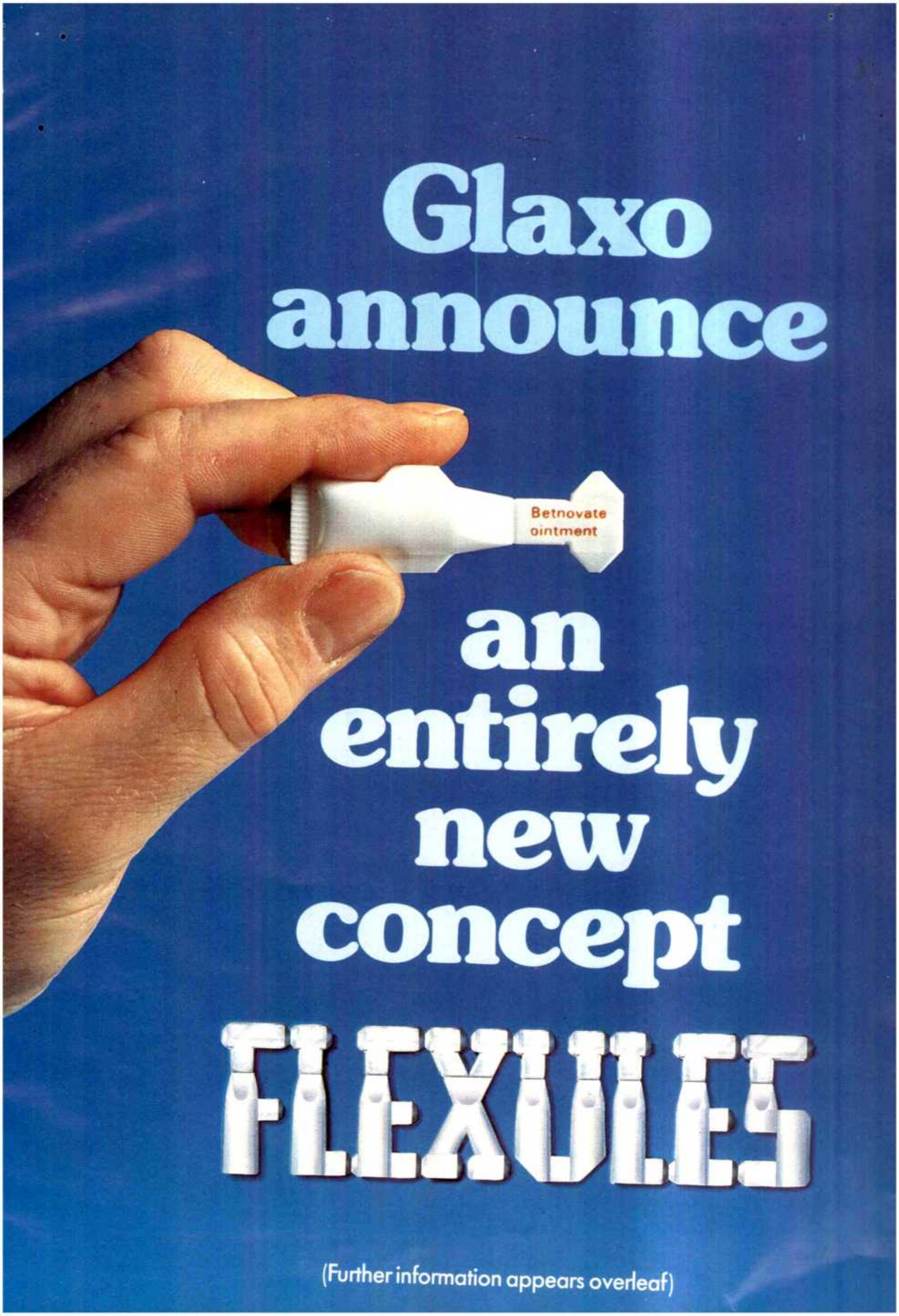


(Further information appears overleaf)

New
rom Glaxo

1 gram single applications of topical steroid creams and ointments.
In packs of 20.

Betnovate Flexules
(**betamethasone valerate**)
Cream and Ointment

Dermovate Flexules
(**clobetasol propionate**)
Cream and Ointment

Eumovate Flexules
(**clobetasone butyrate**)
Cream and Ointment

Efcortelan 1% Flexules
(**hydrocortisone 1%**)
Cream and Ointment

The excellence of the Glaxo topical steroids in the treatment of steroid-responsive dermatoses has been demonstrated in wide clinical use.
Flexules provide units-of-use single applications adding a new dimension of control. In addition to specifying the base and steroid appropriate to the condition you can now be more precise about the
quantity applied and the duration of t
And at the same time reduce contamir
wastage.
Each 1 gram Flexule can generally be
being sufficient to cover lesions appro
the area of an adult forearm.

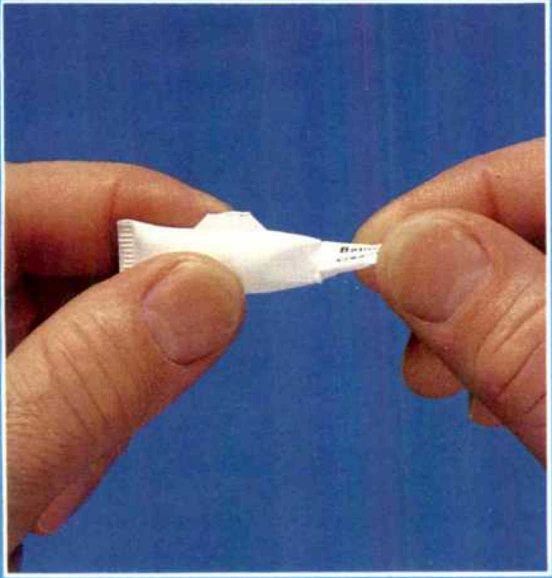
Prescribing information

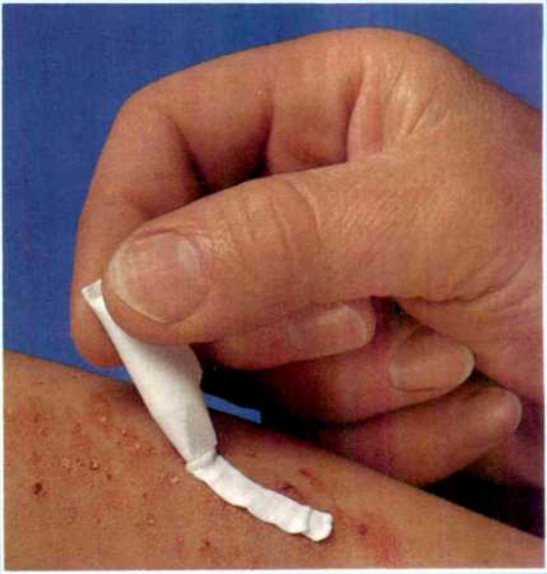
Dosage and administration
Sufficient cream or ointment should be applied to leave the affected area looking glossy. This should be applied up to three times daily depending on the circumstances of the case. However, with Dermovate once or twice daily application is sufficient.

Side effects
General:
With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also, prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism. This is more likely to occur in infants and children, and with occlusion. In infants the napkin may act as an occlusive dressing. Provided the weekly dosage of Dermovate is less
than 50 grams in adults, any pituitary-adrenal suppre
transient with a rapid return to normal values once th
steroid therapy has ceased. The same applies to child
proportionate dosage.
In the unlikely event of signs of hypersensitivity appe
should stop immediately.
Rarely, treatment of psoriasis with corticosteroids (or
thought to have provoked the pustular form of the di

Precautions
Long-term continuous therapy should be avoided, pa
and children in whom adrenal suppression can occur
occlusion. The face, more than other areas of the bo

A new measure of control in topical steroid therapy





In comparison with tube presentations:

Flexules offer the physician

- Greater therapeutic control
- Improved prescribing precision
- More specific patient counselling
- Easier monitoring of patient compliance

Flexules offer the patient

- Treatment that is easy to understand and simple to use
- A convenient compact presentation, hygienic and easy to carry around

Glaxo Leadership and experience in topical steroid therapy

atrophic changes after prolonged treatment with potent topical corticosteroids.

Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy.

With all corticosteroids, prolonged application to the face is undesirable.

Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications

Bacterial, fungal or viral diseases of the skin. Dermovate and Betnovate are also contra-indicated for rosacea, acne and peri-oral dermatitis. Hypersensitivity to the preparation.

Basic NHS cost (exclusive of VAT)	**Product Licence numbers**
Betnovate Cream/Ointment Flexules 20 × 1 gram £3·62	4/5121, 4/5124
Dermovate Cream/Ointment Flexules 20 × 1 gram £4·22	4/0219, 4/0220
Eumovate Cream/Ointment Flexules 20 × 1 gram £3·91	4/0233, 4/0254
Efcortelan Cream/Ointment 1% Flexules 20 × 1 gram £3·19	4/5081, 4/5086

Betnovate, Dermovate, Eumovate, Efcortelan and Flexules are trade marks. Further information is available from:
Glaxo Laboratories Limited, Greenford, Middlesex UB6 0HE

Principles of Clinical Measurement

Formerly *Principles of Measurement for Anaesthetists*

M.K. Sykes M.A. M.B. B.Chir. D.A. F.F.A.R.C.S. F.F.A.R.A.C.S.
M.D. Vickers M.B. B.S. M.R.C.S. L.R.C.P. D.A. F.F.A.R.C.S. F.F.A.R.A.C.S.
C.J. Hull M.B. B.S. F.F.A.R.C.S.

The first edition of this book was uniquely successful as an introductory text for anaesthetists on the basic principles of measurement techniques which could be applied to clinical practice. At that time the importance of the subject to anaesthetists had been recognized by its inclusion in the Primary FFARCS examination, but the practice of clinical measurement was mostly restricted to specialized centres. During the past ten years measurement technology has steadily improved and the use of sophisticated instruments has spread from the cardiac catheter laboratory and operating theatre to the intensive care unit, premature baby unit and obstetric unit. Furthermore, techniques which were at one time mainly the province of the cardiologist, respiratory physiologist or anaesthetist are now increasingly being used by all disciplines, whilst long-term monitoring is frequently practised on the general wards. In preparing a second edition it therefore seemed reasonable to broaden the coverage and to provide a simple text which would be of use not only to anaesthetists, but also to physicians, surgeons, paediatricians and obstetricians. Although aimed primarily at doctors, it is hoped that the text will also be of use to technicians and nurses concerned with clinical measurement or monitoring.

Contents

General principles of measurement
Units of measurement and basic mathematical concepts
Simple electronics
Biological potentials
Data processing and computation
Displaying the signal
Recorders
Electromagnetic radiation and optical measurement
The use of ultrasound in clinical measurement
Measurements using radioactive substances
Patient safety
The measurement of pressure
Direct measurement of intravascular pressures
Indirect methods for measuring arterial pressure
Measurement of gas flow and volume
Measurement of blood flow
Gas and vapour analysis
pH and blood-gas analysis
Thermometry, thermography and humidity measurement
Descriptive and deductive statistics
Inferential statistics

Second Edition, 1980. 320 pages, 227 illustrations
£21.00

Blackwell Scientific Publications

Why mess about with topical antifungals ...when oral Grisovin can be working from within?

For fungal infections, topical therapy is often both messy and inconvenient – and is unlikely to be as completely effective as oral Grisovin.

Grisovin is carried in the bloodstream to the basal layer and gets right to the root of ringworm infections. It protects the newly formed keratin cells from infection and as they move outwards, the fungus is expelled.

During the past 20 years, Grisovin has proved itself in clinical use to be the more effective therapy especially in long established ringworm infections. In addition its oral administration, two 500mg tablets a day, and its relative lack of side effects facilitates patient compliance.

Why mess about with topical antifungals when Grisovin gets right to the root of the problem?

GRISOVIN

griseofulvin

THE INSIDE STORY ON FUNGAL INFECTION

Prescribing information

Dosage and administration

Doses should be taken after meals for maximum absorption.

Adults: normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily. *Children:* usually 10mg/kg (5mg/lb) bodyweight daily in divided doses.

Duration of treatment: for hair or skin at least four weeks' treatment is required, whereas finger or toe nails may need six to twelve months' treatment. Continue for at least two weeks after all signs of infection have disappeared.

Contra-indications

Porphyria or severe liver disease.

Griseofulvin may cause deterioration in some patients with liver disease and liver function should be monitored in such conditions.

Precautions

Griseofulvin may decrease the effect of coumarin anticoagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established, but use during pregnancy is best avoided.

Side effects

Headache, and gastric discomfort sometimes occur, but usually disappear as treatment continues. Rarely, urticarial reactions and erythematous rashes have been reported.

Further information

Grisovin is not effective in infections caused by *Candida albicans* (monilia), Aspergilli, *Malassezia furfur (Pityriasis versicolor)* and Nocardia species.

Basic NHS cost	**Product Licence numbers**
(exclusive of VAT) £9.80	
Bottle of 100 tablets (500mg)	125mg 4/5060 500mg 4/5061

Glaxo

Full prescribing information on Grisovin (trade mark) is available from:
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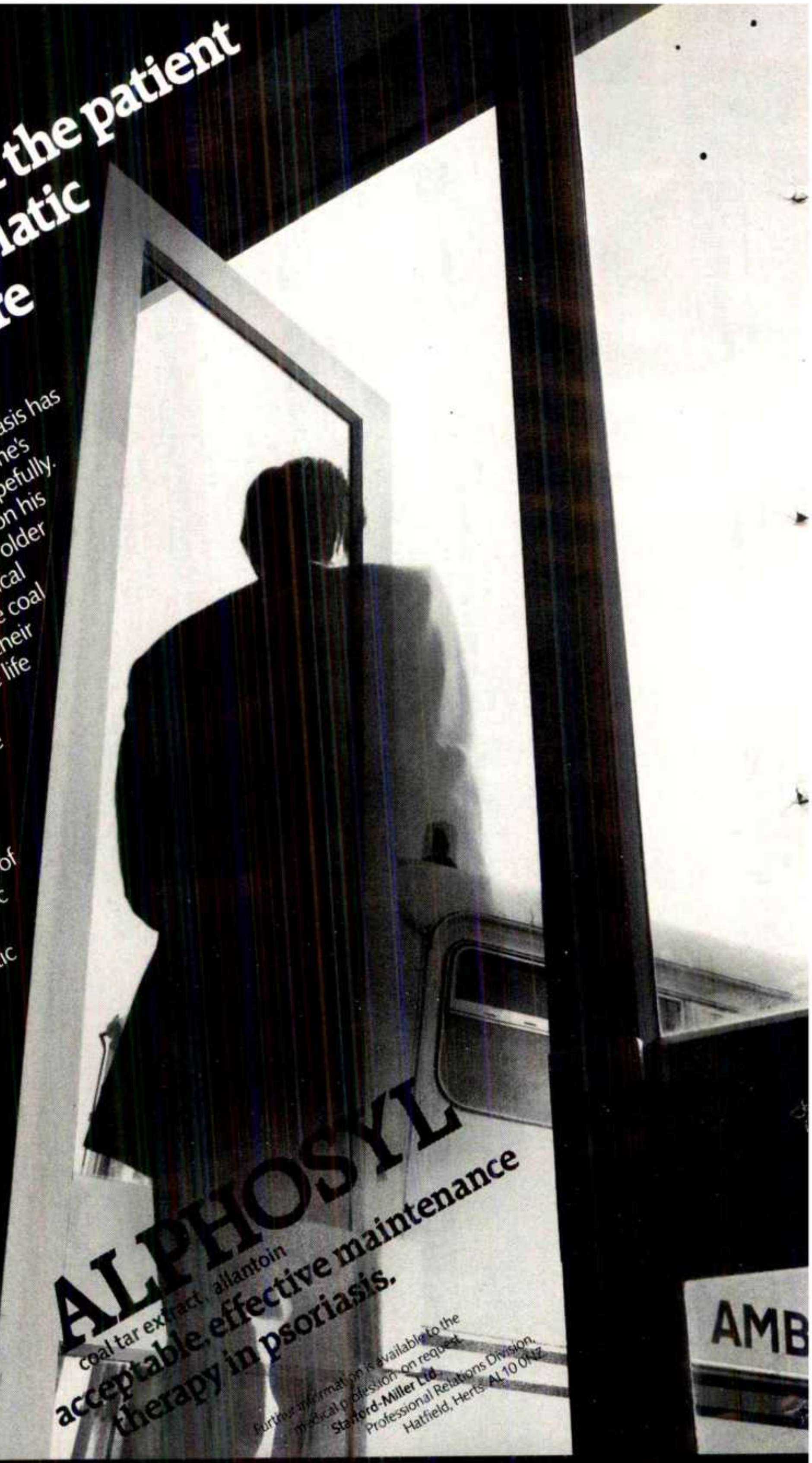
Greenford, Middlesex UB6 0HE

Don't forget the patient
when the psoriatic
eaves your care

Another acute case of psoriasis has
ought under control. Now he's
k to live a normal life. Hopefully.
normal a life will depend on his
nce therapy. Some of the older
admittedly very effective) topical
ents like dithranol and crude coal
tention to themselves by their
ce and smell. It can make life
at home and at work.
Consider a more acceptable
ve: Alphosyl.
phosyl cream and lotion
refined coal tar extract,
ains much of the activity of
al tar without the cosmetic
ks. And Alphosyl also
llantoin to soften psoriatic
d aid in their removal.
use Alphosyl is non-
and free from
able smell, your patient
ore likely to apply it
That means he is less
ave another flare-up.
istress for him, and less
for you.



ALPHOSYL

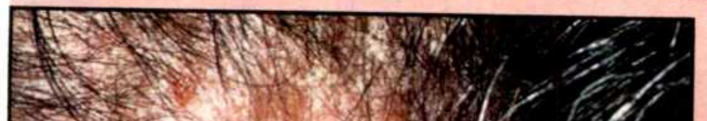
coal tar extract, allantoin

acceptable, effective maintenance therapy in psoriasis.

Further information is available to the medical profession on request.
Stafford-Miller Ltd
Professional Relations Division,
Hatfield, Herts AL10 0NZ

ion LOTION AND CREAM: Containing allantoin 2%, refined alcoholic extract of coal tar 5%, in a greaseless vanishing-cream base. The lotion is a light, free-flowing emulsion and the mooth and homogeneous, light tan in colour. **Uses** Dermatological stimulant for the treatment of psoriasis. **Dosage and Administration** Apply liberally two to four times daily, massage he affected areas. Occlusive dressings are unnecessary. **Contra-indications and Warnings, etc.** For external use only. Discontinue if irritation occurs or in cases of sensitivity to coal tar. **utical Precautions** Do not refrigerate. **Legal Category** No legal restrictions on sale or supply. **Package Quantities** ALPHOSYL LOTION: Bottles of 250ml. ALPHOSYL CREAM: Tube of 60g. **formation** Alphosyl Lotion is the basic treatment for psoriasis of the body and scalp. Alphosyl Cream is designed especially for lubrication in the intertrigenous areas. **Product Licence Nos.**

Common Disorders of the Scalp.



They all have one thing in common — the treatment

1. Psoriasis of the Scalp 2. Seborrhoeic Dermatitis of the Scalp 3. Pityriasis Capitis (Dandruff)

Polytar Liquid

Tar B.P., Cade Oil B.P.C., Coal Tar B.P.C., Arachis Oil extract of crude coal tar, Oleyl alcohol.

Acknowledged as ideal for long-term control.

NEW Polytar Plus with protein

Prescribing Information.

Presentation Polytar Liquid is a concentrated antiseptic tar-medicated scalp cleanser containing the following active ingredients:

Tar B.P.	0.3%
Cade Oil B.P.C.	0.3%
Coal Tar B.P.C.	0.1%
Arachis Oil extract of crude coal tar	0.3%
Oleyl alcohol	1.00%

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
Polytar Liquid	65ml	57p
	150ml	82p
	350ml	£1.48
	1 litre	£3.90
Polytar Plus	150ml	90p

Product Licence Number
Polytar Liquid PL0174/5016 Polytar Plus PL0174/0037

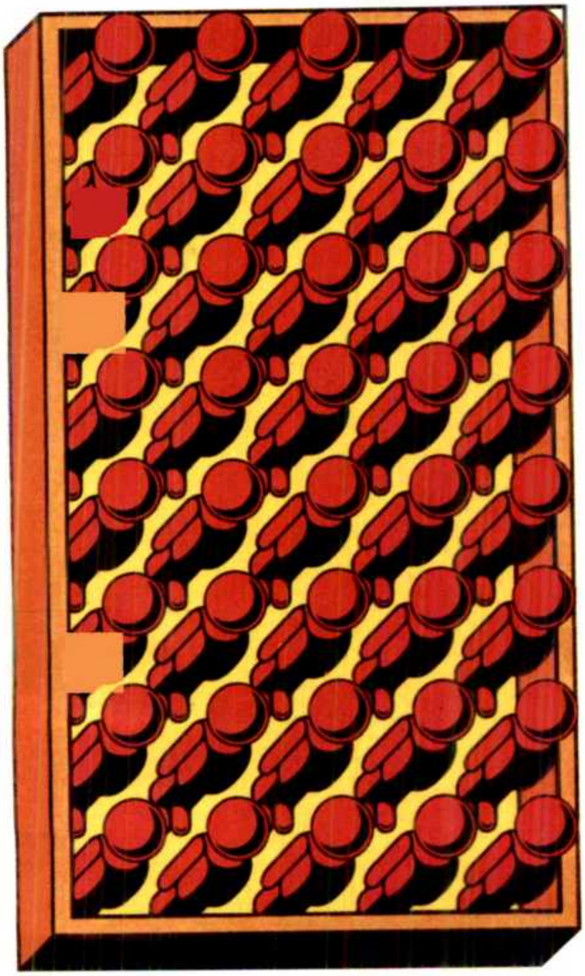
Full information and Data sheet on request.

Stiefel Laboratories (UK) Limited
Wellcroft Road, Slough SL1 4AQ

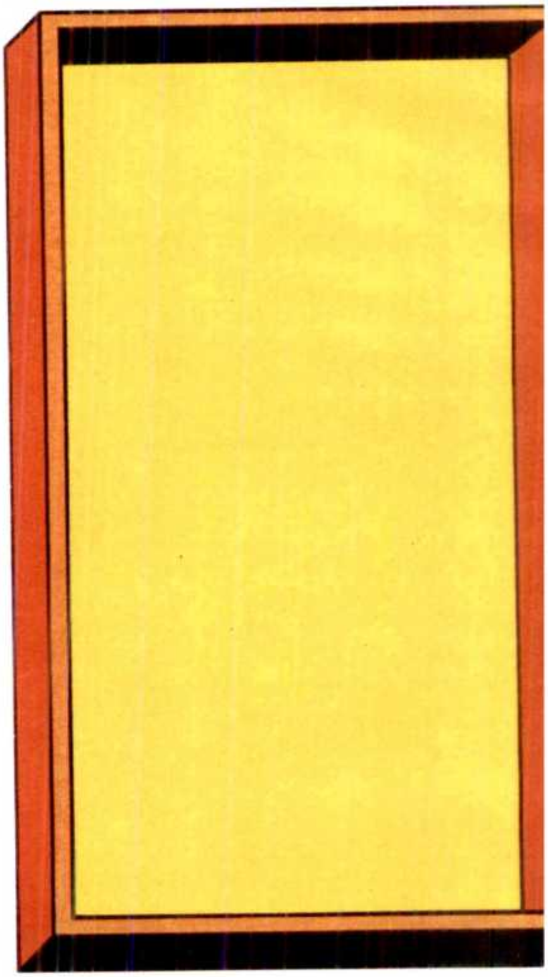
Dithrocream

dithranol BP

Natural selection!



All 40 patients in the trial preferred Dithrocream



Not a single patient preferred dithranol ointm

In a recent clinical trial,[1] Dithrocream 0.25% was compared with dithranol ointment of equivalent strength in the treatment of psoriasis.

Whilst both preparations were effective, there was a unanimous patient preference for Dithrocream with all 40 patients selecting the vanishing cream formulation.

Combining effectiveness, convenience and stability, Dithrocream is the patient's choice for psoriasis treatment.

Presentation

Dithrocream 0.1%: pale yellow aqueous cream containing 0.1% Dithranol B.P.
Dithrocream 0.25%: pale yellow aqueous cream containing 0.25% Dithranol B.P.
Dithrocream Forte: pale yellow aqueous cream containing 0.5% Dithranol B.P.

Indications

For the topical treatment of subacute and chronic psoriasis including psoriasis of the scalp. Dithrocream Forte should only be used for resistant cases.

Directions for use

Apply sparingly to the psoriatic lesions preferably at night and rub in gently and carefully until absorbed. Bathe before the next application. For scalp lesions, shampoo before use. Continue treatment until the skin is smooth and clear.

Contraindications

Acute psoriasis; dithranol sensitivity.

Precautions

Keep away from the eyes. Apply to psoriatic areas only. Use cautiously after recent application of potent steroids. Wash the hands after use.

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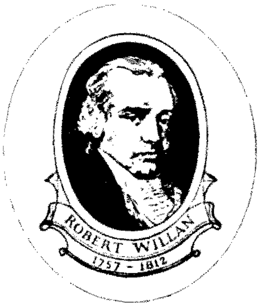

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Contents

NO. 1: JANUARY 1981

Clinical and Laboratory Investigations

PAGE

A method for culturing human hair follicle cells — 1
P.J.J.M.VETERINGS, A.J.M.VERMORKEN AND H.BLOEMENDAL

The histopathology and ultrastructure of liver disease in erythropoietic protoporphyria — 7
M.MACDONALD, DANIELLE GERMAIN AND H.PERROT

Occupational argyria: light and electron microscopic studies and X-ray microanalysis — 19
S.S.BLEHEEN, D.J.GOULD, CHRISTINE I.HARRINGTON, T.E.DURRANT, D.N.SLATER AND J.C.E.UNDERWOOD

The effect of placebo and 'aggro' on the weal and flare response to histamine — 27
L.J.COOK AND SAM SHUSTER

Evaluation of acid α-Naphthyl acetate esterase activity as a marker of T lymphocytes in benign and neoplastic and cutaneous infiltrates — 31
A.C.CHU, E.W.MORGAN AND D.M.MACDONALD

Ultrastructural evidence suggesting an immunomodulatory activity of oral retinoid — 37
D.TSAMBAOS AND C.E.ORFANOS

Pharmacology and Treatment

Plasma 8-methoxypsoralen concentrations in photochemotherapy of psoriasis — 47
L.H.STEVENSON, K.J.A.KENICER, B.E.JOHNSON AND W.FRAIN-BELL

Dapsone in the treatment of lupus erythematosus — 53
THOMAS RUZICKA AND GÜNTER GOERZ

The Duhring chamber assay for corticosteroid atrophy — 57
PETER J.FROSCH, EVA-MARIA REHRENBECK, KELLY FROSCH AND EGON MACHER

Colchicine in the treatment of the cutaneous manifestations of Behçet's disease — 67
YOSHIKI MIYACHI, SHINKICHI TANIGUCHI, MOTOAKI OZAKI AND TAKESHI HORIO

Case Reports

Co-existent atypical bullous pemphigoid and systemic lupus erythematosus — 71
EVA SZABO, SANDO HUSZ AND LASZLO KOVACS

A case of lymphomatoid papulosis and systemic lymphoma — 77
A.G.MESSENGER, T.L.MARSHALL AND R.SUMMERLY

Localized cold reflex urticaria — 83
BEATE M.CZARNETZKI, PETER J.FROSCH AND RAINER SPREKELER

Encephalocraniocutaneous lipomatosis: a new neurocutaneous syndrome — 89
NESTOR P.SANCHEZ, ARTHUR R.RHODES, FRED MANDELL AND MARTIN C.MIHM

Society Proceedings

Proceedings of the 219th Meeting of The Netherlands Society for Dermatology and Venereology — 97

Book Reviews — 111

News and Notices — 115

NO. 2: FEBRUARY 1981

Clinical and Laboratory Investigations

PAGE

Human dermal microvasculature: I. Its segmental differentiation. Light and EM study — 117
J.C.HIGGINS AND R.A.J.EADY

Skin lesions in angioimmunoblastic lymphadenopathy: histological and immunological studies — 131
M.G.BERNENGO, L.LEVI AND G.ZINA

T lymphocytes and mononuclear phagocytes in the skin infiltrate of systemic and discoid lupus erythematosus and Jessner's lymphocytic infiltrate 141
Y.T.KONTTINEN, S.REITAMO, A.RANKI AND M.SEGERBERG-KONTTINEN
Adid glycosidases in basal cell epithelioma and in seborrhoeic keratosis 147
J. DE BERSAQUES
Follicular psoriasis 153
L.STANKLER AND S.W.B.EWEN
Repeated patch testing in penicillin allergy 157
D.P.BRUYNZEEL AND W.G.VAN KETEL
The influence of ultraviolet radiation on allergic contact dermatitis in the guinea-pig. I. UVB radiation 161
WARWICK L.MORISON, JOHN A.PARRISH, MICHAEL E.WOEHLER AND KURT J.BLOCH
The influence of ultraviolet radiation on allergic contact dermatitis in the guinea-pig. II. Psoralen/UVA radiation 165
WARWICK L.MORISON, JOHN A.PARRISH, MICHAEL E.WOEHLER AND KURT J.BLOCH
Isolation and biochemical characterization of tyrosinase-rich GERL and coated vesicle in melanin synthesizing cells 169
GENJI IMOKAWA AND YUTAKA MISHIMA

Pharmacology and Treatment

Halogenation and topical corticosteroids: a comparison between the 17-butyrate esters of hydrocortisone and clobetasone in ointment bases 179
C.F.ALLENBY AND C.G.SPARKES
Factitious urticaria (dermographism): treatment by cimetidine and chlorpheniramine in a randomized double-blind study 185
SURRINDER KAUR, MALCOLM GREAVES AND NAHID EFTEKHARI
Topical treatment of recurrent herpes simplex (HS) and post-herpetic erythema multiforme (PHEM) with low concentrations of zinc sulphate solution 191
ISSER BRODY

Case Reports

Report of an infant with GM- gangliosidosis type I and extensive and unusual mongolian spots 195
MARC WEISSBLUTH, NANCY B.ESTERLY AND WILLIAM A.CARO
Dapsone and severe hypoalbuminaemia in dermatitis herpetiformis 201
R.E.COWAN AND J.T.WRIGHT

Brief Communications

Ketotifen and terbutaline in urticaria 205
ERIC M.SAIHAN
Serum immunoglobulins in lichen planus 207
JOHN M.MAHOOD

Review Article

Cutaneous manifestations of paraproteinaemia—part II 209
E.RUSSELL JONES

Correspondence 221

Book Reviews 223

International Committee of Dermatology Meeting 227

News and Notices 229

NO. 3: MARCH 1981

PAGE

Clinical and Laboratory Investigations

The kinetics of metaphase arrest in human psoriatic epidermis: an examination of optimal experimental conditions for determining the birth rate 231
IAN RALFS, RODNEY DAWBER, TERENCE RYAN, MARK DUFFILL AND NICHOLAS A.WRIGHT

An attempt to use vincristine and colcemid to measure proliferative rates in normal human epidermis in vivo 243
R.S.CAMPLEJOHN, S.GELFANT AND DAN CHALKER

Pityriasis rubra pilaris: epidermal cell kinetics 249
I.G.RALFS, R.P.R.DAWBER, T.J.RYAN AND N.A.WRIGHT

Pityriasis rubra pilaris and retinol-binding protein 253
ALDO F.FINZI, GIANFRANCO ALTOMARE, LUIGI BERGAMASCHINI AND ANTONIA TUCCI

The relevance of morphometry in the differential diagnosis of cutaneous T cell lymphomas 257
E.M. VAN DER LOO, W.A. VAN VLOTEN, C.J.CORNELISSE, E.SCHEFFER AND C.J.L.M.MEIJER

Flow cytometry as a tool for the study of cell kinetics in skin 2. Cell kinetic data in psoriasis 271
F.W.BAUER, N.H.C.N.CROMBAG, J.B.M.BOEZEMAN AND R.M. DE GROOD

Autoradiographic investigations on the cell kinetics of epidermis and periderm of limb buds from mouse embryos *in vitro* 277
R.HERKEN AND U.SCHULTZ-EHRENBURG

Factitious urticaria: Red Dermographism 285
ROBERT P.WARIN

Further evidence for increased light sensitivity in patients with malignant melanoma 289
HARRY BEITNER, ULRIK RINGBORG, GORAN WENNERSTEN AND BENGT LAGERLOF

Eye protection in psoralen photochemotherapy 295
J.B.DAVEY, B.L.DIFFEY AND J.A.MILLER

Cyclic GMP metabolism in psoriasis: increased activity of soluble epidermal cyclic GMP phosphodiesterase and its modulation by calcium 301
JOHN S.CANTIERI, GUSTAV GRAFF AND NELSON D.GOLDBERG

Epidemiology of congenital pigmented naevi: 1. Incidence rates and relative frequencies 307
EDUARDO E.CASTILLA, MARIA DA GRACA DUTRA AND IEDA M.ORIOLI-PARREIRAS

HLA antigens in patients with scabies 317
EDVARD S.FALK AND ERIK THORSBY

HLA-DR3 in dermatitis herpetiformis 321
HUBERT PEHAMBERGER, KARL HOLUBAR AND WOLFGANG R.MAYR

Pharmacology and Treatment

A clinical evaluation of the effects of an aromatic retinoid (Tigason), combination of retinoid and PUVA, and PUVA alone in severe psoriasis 325
J.LAUHARANTA, T.JUVAKOSKI AND A.LASSUS

Case Report

Primary lymphoblastic non-Hodgkin's lymphoma of the skin 333
R.WILLEMZE, W.A.VAN VLOTEN, E.M.VAN DER LOO AND C.J.L.M.MEYER

Brief Communication

Alopecia areata regrowth induced by *Primula obconica* 339
E.J.RHODES, W.DOLMAN, C.KENNEDY AND R.R.TAYLOR

Comment

Changing concepts in melanoma management 341
F.RAMPEN

Correspondence 349

Book Reviews 355

Obituaries 358

News and Notices 359

NO. 4: APRIL 1981

Clinical and Laboratory Investigations

PAGE

Recurrent urticaria: clinical investigation of 330 patients 369
LENNART JUHLIN

Hepatitis B surface antigen deposition in the blood vessel walls of urticarial lesions in acute hepatitis B 383
H.A.M.NEUMANN, P.J.M.BERRETTY, S.C.C.REINDERS FOLMER AND R.H.CORMANE

Distribution and degradation of albumin in extensive skin disease 389
A.-M.WORM, E.TAANING, N.ROSSING, H.-H.PARVING AND O.J.CLEMMENSEN

Photosensitization of *Trichophyton* with 8-methoxypsoralen to UV-A light radiation 397
TAKESHI HORIO AND SHOHEI WATANABE

In vivo effects of PUVA on lymphocyte function 405
WARWICK L.MORISON, JOHN A.PARRISH, KURT J.BLOCH AND JOEL I.KRUGLER

Some factors affecting prognosis in pemphigus vulgaris and pemphigoid 415
J.A.SAVIN

Epidemiology of congenital pigmented naevi: II. Risk factors 421
EDUARDO E.CASTILLA, MARIA DA GRAÇA DUTRA AND IDÊA M.ORIOLI-PARREIRAS

Incorporation of D-(^{3}H)glucosamine into normal and psoriatic epidermal glycoconjugates 429
IAN A.KING, ANNE TABIOWO AND C.J.PAUL

Sulphhydryl and stainings of subepidermal hyaline bodies 437
KIICHIRO DANNO AND TAKESHI HORIO

Pharmacology and Treatment

Serum levels of trimethylpsoralen after oral administration 443
L.STOLK, A.H.SIDDIQUI AND R.H.CORMANE

Serum and saliva levels of 8-methoxypsoralen after rectal administration as a micro-enema 447
L.STOLK, A.H.SIDDIQUI, A.KAMMEYER, R.H.CORMANE AND P.A.VAN ZWIETEN

Inhibition of lipase production in *Propionibacterium acnes* by sub-minimal-inhibitory concentrations of tetracycline and erythromycin 453
G.F.WEBSTER, K.J.MCGINLEY AND J.J.LEYDEN

Case Reports

Zinc deficiency with transitory acrodermatitis enteropathica in a boy of low birth weight 459
INGA BLOM, STEN JAMESON, FREDRIK KROOK, BIRGITTA LARSSON-STYMNE AND OARS WRANNE

Acne induced by dantrolene 465
A.C.PEMBROKE, S.R.SAXENA, M.KATARIA AND K.D.ZILKHA

Richner-Hanhart syndrome (tyrosinaemia-II) (report of four cases without ocular involvement) 469
A.REHÁK, M.M.SELIM AND G.YADAV

Demethylchlortetracycline and griseofulvin as examples of specific treatment for mycosis fungoides 477
WALTER B.SHELLEY

Brief Communications

Erythema multiforme during treatment with mianserin—a case report 481
EHSANULLAH QURAISHY

Oral zinc sulphate in alopecia areata—a double blind trial 483
R.D.EAD

Comment

The specificity of early changes in the skin during carcinogenesis 485
M.C.BIBBY

Book Reviews 489

News and Notices 493

NO. 5: MAY 1981

Clinical and Laboratory Investigations

PAGE

Discoid lupus erythematosus-like lesions and stomatitis in female carriers of X-linked chronic granulomatous disease 495
FLEMMING BRANDRUP, CHRISTIAN KOCH, MICHAEL PETRI, MORTEN SCHIØDT AND STAEHR JOHANSEN

Loss of β_2 microglobulin from the cell surface of cutaneous malignant and premalignant lesions 507
MARLYN L.TURBITT AND RONA M.MACKIE

The presence of β_2 microglobulin on the membrane of the keratinocyte in premalignant skin disorders 515
H.R.KORTHALS ALTES-LEVY VAN VINNINGHE AND H.A.M.NEUMANN

Human dermal microvasculature: II. Enzyme histochemical and cytochemical study 521
J.C.HIGGINS AND R.A.J.EADY

Biochemical characterization of tyrosinase inhibitors using tyrosinase binding affinity chromatography 531
GENJI IMOKAWA AND YUTAKA MISHIMA

An action spectrum for 8-MOP induced sunburn cells in mammalian epidermis 541
A.R.YOUNG AND I.A.MAGNUS

Absorption of ultraviolet radiation by skin surface lipid 549
P.C.BEADLE AND J.L.BURTON

Herpes gestationis and anti-HLA immunization 553
E.EBERST, M.M.TONGIO, B.EBERST, S.MAYER AND A.BASSET

The association between lichen sclerosus et atrophicus and HLA-B40 561
CHRISTINE I.HARRINGTON AND K.GELSTHORPE

An investigation into the incidence of auto-immune disorders in patients with lichen sclerosus and atrophicus 563
CHRISTINE I.HARRINGTON AND I.R.DUNSMORE

Case Reports

Idiopathic pyoderma gangrenosum and impaired lymphocyte function: failure of azathioprine and corticosteroid therapy 567
S.M.BREATHNACH, G.C.WELLS AND H.VALDIMARSSON

Porphyria cutanea tarda (PCT) in a patient with chronic renal failure on haemodialysis 575
JACK R.LICHTENSTEIN, ELIZABETH J.BABB AND BERTRAM F.FELSHER

Porphyria cutanea tarda in a haemodialyzed patient 579
G.C.TOPI, GANDOLFO L.D'ALESSANDRO, G.C.CANCARINI, F.DE COSTANZA, D.GRISO AND M.RAVELLI

Cutaneous ultrastructural diagnosis of ceroid-lipofuscinosis 581
MASAMITSU ISHII, KUNIAKI TAKAHASHI, TOSHIO HAMADA, AKEMI TANAKA AND SHINOBU HIGAMI

Cerebriform tongue—a clinical sign in pemphigus vegetans 587
S.PREMALATHA, S.JAYAKUMAR, PATRICK YESUDIAN AND A.S.THAMBIAH

Cerebellar ataxia and sensory neuropathy associated with dermatitis herpetiformis 593
TAWFIQUE K.DANESHMEND AND CHRISTOPHER GARDNER-THORPE

Brief Communications

Unsuccessful treatment of alopecia areata with dapsone 597
P.S.FRIEDMANN

Juvenile plantar dermatosis in a adult 599
D.B.CZARNECKI, P.S.COWEN AND T.J.CONNORS

Correspondence 607

Book Reviews 609

News and Notices 610

NO. 6: JUNE 1981

Clinical and Laboratory Investigations

PAGE

Dermatological non-disease: a common and potentially fatal disturbance of cutaneous body image 611
J.A.COTTERILL

P. 25 449

Autoantibodies to an epidermal protease inhibitor in skin diseases 621
V.K.HOPSU-HAVU, KIRSTI KALIMO AND M.JÄRVINEN

Measurement of biological ageing in human epidermis 627
R.MARKS

Studies on the plasma membrane of normal and psoriatic keratinocytes. 3. Uptake of labelled sugars and their incorporation into glycoconjugates 635
H.ROELFZEMA, M.BERGERS, P.E.J. VAN ERP, J.M.GORMANS AND P.D.MIER

Lectin binding studies on suspensions of isolated epidermal keratinocytes 641
J.M.GOMMANS AND J.J.M.A. VAN DEN HURK

Synthesis and turnover of membrane glycoconjugates in monolayer culture of pig and human epidermal cells 649
HELEN W.DAVIES AND M.D.TROTTER

Molecular basis of substrate and inhibitory specificity of tyrosinase: phenolic compounds 659
S.PASSI AND M.NAZZARO-PORRO

Pharmacology and Treatment

Severe disorders of keratinization effects of treatment with Tigason (etretinate) 667
RONALD MARKS, ANDREW Y.FINLAY AND PETER J.A.HOLT

The efficacy of an aromatic retinoid, Tigason (etretinate), in the treatment of Darier's disease 675
S.M.BURGE, J.D.WILKINSON, A.J.MILLER AND T.J.RYAN

Low-dose PUVA maintenance in psoriasis following Ingram therapy 681
P.C.M. VAN DE KERKHOF AND J.W.H.MALI

Effect of topical 17 propyltestosterone on sebum excretion in man 685
FERGUS LYONS AND SAM SHUSTER

Case Reports

Sclerosing lymphangitis of the penis: a lymphangiofibrosis thrombotica occlusiva 687
WOLFGANG CH.MARSCH AND GÜNTER STÜTTGEN

Allergic contact dermatitis due to an epoxy acrylate 697
JAMES R.NETHERCOTT

Cold urticaria with persistent weals 705
LENNART JUHLIN

Brief Communications

An unusual adverse reaction to Ro 10-9359 709
K.MOULOPOULOU-KARAKITSOU

UV-induced pyrimidine dimers and rosacea 711
ENRICO NUNZI, ALFREDO REBORA, JAN J.CORNELIS AND RUDY H.CORMANE

Review Article

Mixed connective tissue disease 713
C.BLACK

Correspondence 721

Book Reviews 723

Editorial 729

News and Notices 730

Index 733

British Journal of Dermatology (1981) **104**, 117.

Clinical and Laboratory Investigations

Human dermal microvasculature: I. Its segmental differentiation. Light and electron microscopic study*

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Accepted for publication 17 April 1980

SUMMARY

Light microscopic examination of 1 μm thick epoxy resin sections of human skin preserved in fixative normally used for electron microscopy allows division of the microvasculature of the reticular and superficial dermis into several distinct segments. More precise delineation of these components can be achieved by correlative study using electron microscopy.

Measurement of vessel diameter in tissue sections is of limited value in vessel classification because there is a wide overlap between different vascular segments and the results are influenced by the type of fixative used for processing the tissue. Detailed observation of the morphological characteristics of the endothelium and its investment, including the elastic and basal laminae, is considered preferable for identifying individual vascular segments.

Differences in body region do not appear to have any obvious influence on vessel structure although there are regional differences in vessel density and arrangement.

In comparison with earlier studies on animal tissues or human skin, no structures resembling arteriovenous shunts or precapillary sphincters were observed in the present investigation. However, closed fenestrae and other structures thought to be transendothelial channels were often found at the tips of capillary loops adjacent to the epidermis.

Previous accounts of the ultrastructure of human skin microvasculature include those of Hibbs, Burch & Phillips (1958), Odland (1961), Macher & Vogel (1962), Friederici, Tucker & Schwartz

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* Parts of this work were presented at a meeting of the Investigative Group of the British Association of Dermatologists held in Glasgow on January 18–19, 1980, and at a meeting of the British Microcirculation Society, held in London on April 6–7, 1979.

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(1966), Zelickson (1966), McLeod (1970), Seifert & Klingmüller (1972) and Takada & Hattori (1972). Only Yen & Braverman (1976) and Braverman & Yen (1977), however, have attempted systematically to identify the vessel segments comprising the superficial dermal network as a whole.

During the routine examination of skin blood vessels and in an investigation to assess the effects of different fixatives on their ultrastructure, we noted several points of difference from the above accounts. We therefore set out to re-examine by light and electron microscopy the different segments of normal human dermal microvasculature to provide a basis for future experimental studies. In this report, our findings relate chiefly to the superficial dermal vascular plexus and associated intrapapillary capillary loops. Observations on vessels in deep dermis or in networks around epidermal appendages are not included.

MATERIALS AND METHODS

Skin samples from various regions (including scalp, forehead, below the ear, cheek, lip, chin, back, upper arm, forearm, wrist, hand, scrotum, thigh, calf and toe) of twenty-five individuals, eighteen males and seven females, aged 19–85 years, were examined. Several samples were collected during the local excision of various cutaneous lesions for diagnostic or therapeutic purposes when the surrounding clinically normal skin was used. In these cases the skin was infiltrated with lignocaine hydrochloride (10·6 μg/ml) and adrenaline (5 μg/ml). Otherwise, biopsies of normal skin were taken without anaesthesia from healthy volunteers using a highspeed electric punch of 4 mm diameter. The specimens were divided into small pieces, each not more than 3 mm^3, and fixed by immersion using one of the following techniques:

(a) Fixation in 1·33% osmium tetroxide in S-collidine buffer (pH 7·35) for 2 h on ice.

(b) Primary fixation in half-strength Karnovsky fixative (Karnovsky, 1965) in 0·1 M cacodylate buffer (pH 7·4) with 0·05% $CaCl_2$ for 4 h at room temperature, rinse in 0·067 M cacodylate buffer and post-fixation in osmium tetroxide as in (a).

(c) Primary fixation in 5% glutaraldehyde in 0·1 M cacodylate buffer for 1 h on ice, rinse in 0·1 M cacodylate buffer followed by osmium tetroxide fixation as in (a).

(d) Primary fixation in a solution containing 1·5% formaldehyde, 1·5% glutaraldehyde and polyvinylpyrrolidone as described by Forssmann *et al.* (1977) at pH 7·35 for 1–3 h on ice, followed by storage in a second fixative similar to the first but with the addition of picric acid (Forssmann *et al.*, 1977) and by post-fixation in 1% osmium tetroxide in phosphate buffer (pH 7·4) (Millonig, 1962) for 2 h on ice. For comparison some of these tissue samples were post-fixed in osmium tetroxide as in (a).

After dehydration in ethanol and propylene oxide, the tissues were embedded in Epon. Semi-thin (1 μm) sections cut perpendicular to the skin surface were stained with methylene blue and basic fuchsin (Huber, Parker & Odland, 1968) and examined by light microscopy. Ultra-thin sections were cut with diamond knives on an LKB I ultramicrotome, collected on uncoated grids or grids coated with parlodion and carbon, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined in an AEI EM6B electron microscope.

Light microscope measurements of vessel diameter were made with an eye-piece graticule on semi-thin sections of tissues fixed by methods (a), (b) and (d). For each vessel segment identified according to the criteria given below, twenty measurements were made of the luminal, endothelial tube and outside diameters (Fig. 1). Wherever possible measurements were made upon vessels cut in cross section.

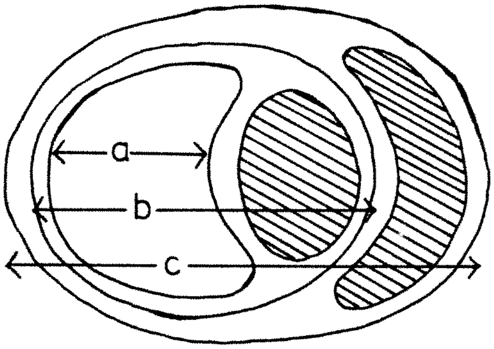


FIGURE 1. Diagram showing the various measurements of vessel diameter obtained by light microscopy: (a) luminal diameter, (b) endothelial tube diameter, (c) outside diameter.

RESULTS

Light-microscopic observations

Observations were confined to vessels underlying inter-follicular epidermis; the vascular network surrounding the appendages was not studied systematically. The microvasculature of mid- and upper dermis could be divided into the following nine segments (Figs 2–8): ascending arteriole, arteriole of superficial plexus, terminal arteriole, arterial and venous side of the capillary loop, postcapillary venule, venule of superficial plexus (upper and deep components interconnected by vertical shunts), and descending collecting venule. Apart from the distinct capillary loop unit there were no sharp divisions between the different vessel segments. Identification of vessel type was initially based on the location of the vessel within the section, and usually necessitated examination of serial sections to follow the vessel through to its origin or termination; this was particularly true for terminal arterioles and postcapillary venules. The three-dimensional arrangement of blood vessels within the mid- and superficial dermis was determined by examining many serial sections (up to 270 μm from any one block).

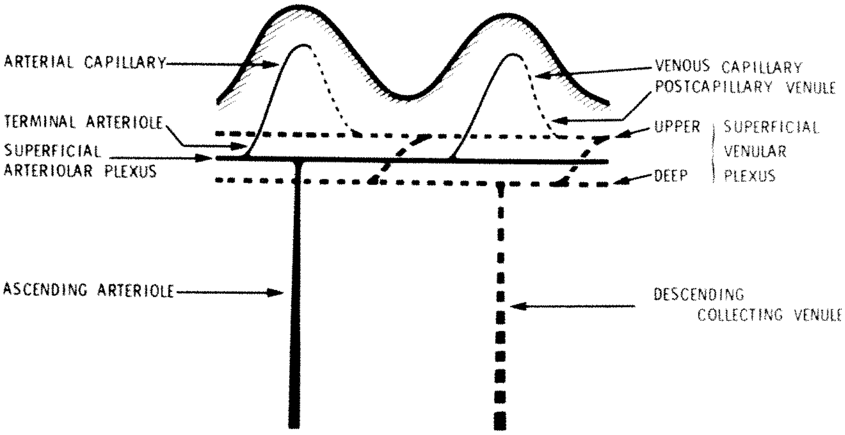
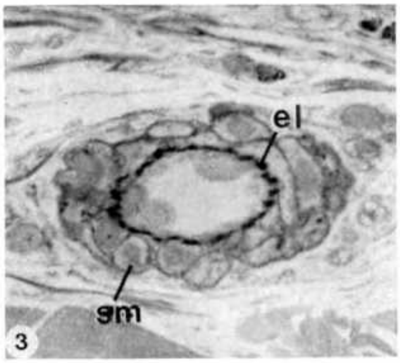
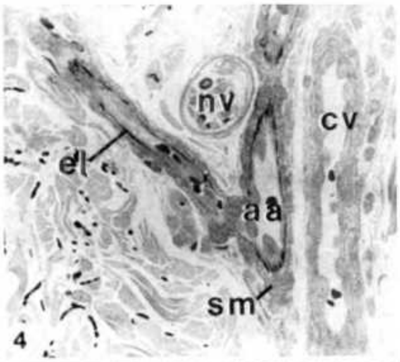
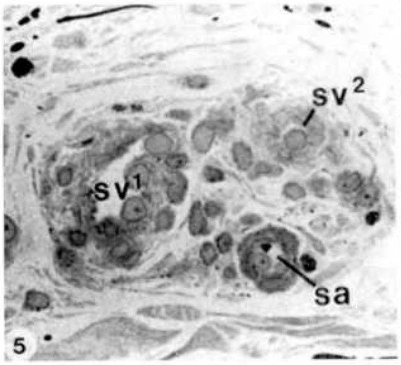
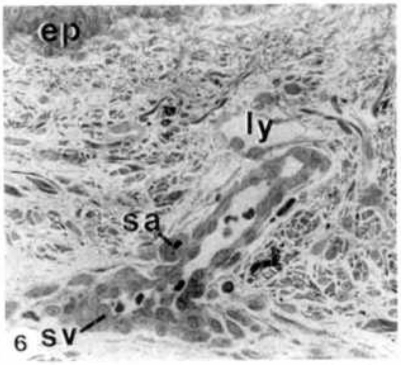


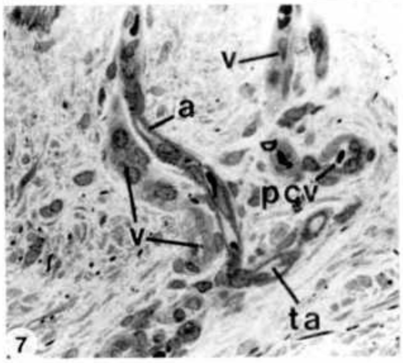
FIGURE 2. Diagram showing segmental arrangement of superficial dermal microvasculature.

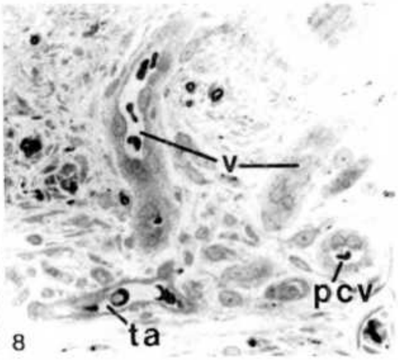












FIGURES 3–8 are light micrographs of 1 μm Epon sections stained with methylene blue and basic fuchsin.

FIGURE 3. Cross section of a large ascending arteriole in the deep dermis. Note that 1–2 layers of smooth muscle cells (sm) and an elastic lamina (el) surround the endothelium (× 600).

FIGURE 4. A branching ascending arteriole (aa), a descending collecting venule (cv), and a nerve (nv) in the mid-dermis. The ascending arteriole is distinguished by the plump smooth muscle cells (sm), elastic lamina (el) and elongated endothelial nuclei. The endothelial cells of the descending collecting venule have rounded nuclei and bulge into the lumen (× 280).

FIGURE 5. Cross section of vessels in the deep component of the superficial venular plexus (sv^1, sv^2) and superficial arteriolar plexus (sa). Note larger dimensions of the venule (sv^1) and its irregular luminal surface (× 600).

FIGURE 6. Venule of the deep component of the superficial plexus (sv) branching to form a vertical shunt (which in serial sections was shown to connect with the upper component of the plexus). sa, arteriole of superficial plexus; ly, lymphatic; ep, epidermis (× 250).

FIGURES 7–8. Terminal arteriole (ta), arterial (a) and venous (v) side of a capillary loop, and postcapillary venule (pcv) in the superficial dermis. The arterial vessels have elongated endothelial nuclei and thin, amorphous periendothelial matrix compared to the rounded endothelial nuclei and thick periendothelial matrix of the venous vessels. Also note that the arterial vessels have a thinner outside diameter compared to the venous vessels (Fig. 7 × 330. Fig. 8 × 370).

Confirmation of vessel type was based upon a number of structural characteristics. Those peculiar to arterial segments (Figs 3–5 and 7) included elongated endothelial cell nuclei, a relatively smooth luminal surface and a thin blue-green staining perivascular matrix. The one exception was the larger ascending arterioles of the mid-dermis, where the endothelial nuclei were often rounded in cross section. In contrast, venous vessels (Figs 4–6 and 8) generally had rounded, indented nuclei which caused the endothelial cells to bulge into the lumen, an irregular luminal surface and a thicker, more easily resolved perivascular matrix. The endothelium at the very tip of the papillary loops was usually attenuated. Ascending arterioles could be easily distinguished from other vessels by the presence of an internal elastic lamina (Figs 3 and 4) which became more sparse with reduction in vessel size and disappeared at the level of the superficial plexus. Single or multiple layers of smooth muscle cells were always found in the walls of ascending arterioles, and often in arterioles of the superficial plexus and descending collecting venules in the mid-dermis. These were replaced by a continuous sheath of pericytes in terminal arterioles, postcapillary venules and venules of the superficial plexus. Capillaries were invested by a single discontinuous layer of pericytes.

The results of vessel measurements using one fixation technique are shown in Fig. 9. These represent the pooled results from all body regions studied, since preliminary light microscope measure-

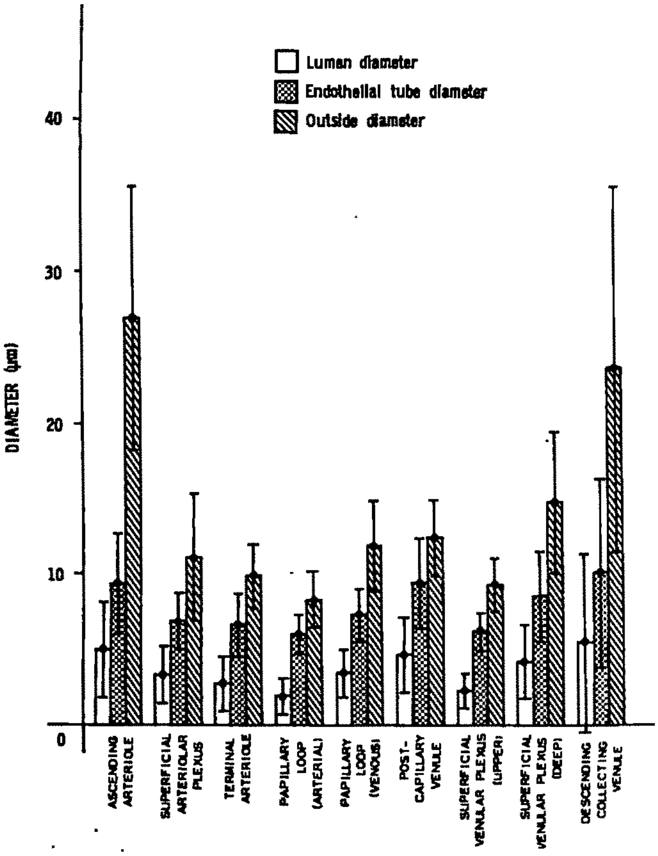


FIGURE 9. Histogram showing diameters of different segments of dermal microvasculature. Fixation after Forssmann *et al.* (1977). Each column represents the mean of twenty observations. The vertical lines indicate the s.d.

ments did not reveal any difference in vessel diameters between different regions. The adrenaline used with the local anaesthetic during the collection of some of the samples had no obvious effect on blood vessel diameter. A comparison of the means of twenty vessel diameter measurements in tissue taken from adjacent sites in the arm of the same subject (a) with both lignocaine and adrenaline and (b) with lignocaine but without adrenaline, showed no statistical difference (Student's t-test).

Treatment of the initial readings of vessel diameter (luminal, endothelial tube and outside diameters treated separately) with Analysis of Variance enabled an estimate to be made of: (1) the variance within the twenty replicates, (2) the variance between the nine vessel categories and (3) the variance due to the type of fixative used (methods a, b and d). The tests revealed that the variance within vessel groups was not significant, but there was a significant difference in vessel diameter both between different vascular segments ($P<0{\cdot}01$) and as a result of different methods of fixation ($P<0{\cdot}01$). The variance between the nine vessel categories contributed most to the overall variance.

Electron-microscopic observations

The best preservation of blood vessel ultrastructure was obtained by method (d) (Forssmann *et al.*, 1977) (Fig. 14). Tissues preserved in this manner appeared relatively artifact-free whereas those fixed by osmium tetroxide alone (method (a)) often showed swelling of the endothelial cytoplasm and organelles. Fixation in half-strength Karnovsky solution (method (b)) caused even greater swelling resulting in large balloons of cytoplasm (which often appeared burst) extending into the lumen, and a general disorganization of the cell structure, including the occurrence of wide spaces between the nucleus and cytoplasm. Glutaraldehyde fixation (method (c)) on the other hand resulted in a general shrinkage of cells and an apparent reduction in the number of endothelial pinocytotic vesicles.

Endothelial cells were similar in all segments of the microvasculature in that they contained mitochondria, ribosomes, Golgi apparatus and associated vesicles with a diameter of 70±15·27 (mean±s.d.) nm, both rough and smooth endoplasmic reticulum, centrioles, primary and secondary lysosomes and multivesicular bodies. Weibel–Palade bodies (Weibel & Palade, 1964) were sparse in terminal arterioles and arterial capillaries but more abundant in the venous vessels (Figs 11 and 14). Both smooth (diameter = 75±9·01 nm) and coated (diameter = 150±116·5 nm) types of pinocytotic vesicles were present (Fig. 10) although the former were more abundant. The vesicles opened onto the luminal, abluminal and lateral surfaces of endothelial cells and the smooth vesicles occasionally formed branching chains or channels extending into the cytoplasm from the surface. In a single terminal arteriole and in many tips of capillary loops, transendothelial channels were formed by a single vesicle, or a series of vesicles having fused together, opening into two sides of the cell (Fig. 13b, c and d). The numbers of pinocytotic vesicles opening on the luminal surface were noticeably less in arterioles compared with venules. At the very tips of all capillary loops examined from different body regions, closed fenestrae (57·74±5·98 nm in diameter) were observed on that side of the vessel adjacent to the overlying epidermis (Fig. 13 and 13a).

Filaments 7–10 μm thick were present in the endothelium in variable amounts, and in general were more abundant in venous vessels (Fig. 14). They were distributed in disorganized arrays throughout the cytoplasm or were arranged in bundles, parallel to the plasma membrane, in the perinuclear region. Small dense areas, resembling the dense bodies of smooth muscle cells, were regularly seen against the abluminal endothelial membrane of the larger arterioles (Fig. 10). These areas were associated with bundles of fine actin-like filaments (5–6 nm in diameter). The luminal and abluminal surfaces of endothelial cells were more irregular in the venous than in the arterial side (Fig. 14). Structures resembling tight and gap junctions were found between endothelial cells although reliable identification was often difficult due to the obliqueness of many sections.

The elastic lamina was present in arterioles as cords (up to 4 μm in diameter), running longi-

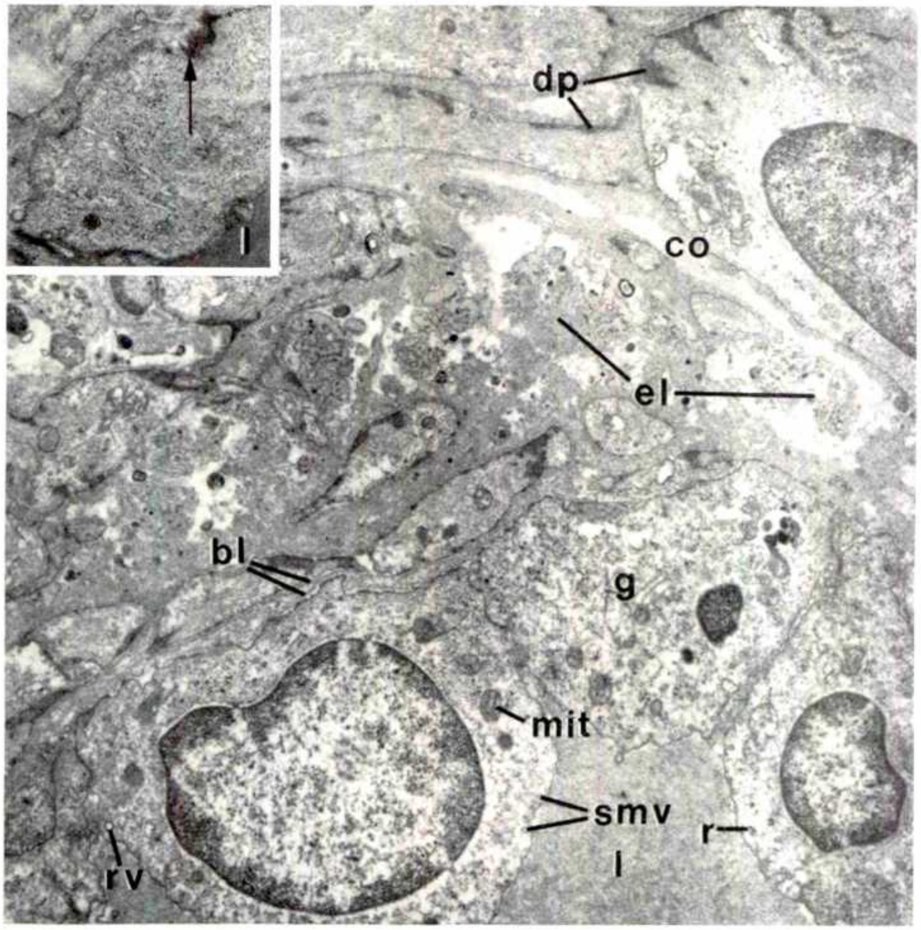


FIGURE 10. Electron micrograph showing part of the wall of an ascending arteriole in the mid-dermis. Smooth-surfaced (smv) and rough-surfaced (rv) vesicles, mitochondria (mit), ribosomes (r) and Golgi apparatus (g) can be seen in the endothelial cells. The basal lamina (bl) closely surrounds both endothelial and smooth muscle cells and merges with a matrix of similar electron density, which envelopes all components of the vessel wall. The elastic lamina (el) is found between the endothelial cells and smooth muscle cells or between the smooth muscle cells themselves. There are several layers of smooth muscle cells with characteristic dense plaques (dp). Collagen (co) is associated with the elastic lamina. l, lumen. (× 5,500). Insert shows a small dense area (arrowed), resembling a dense plaque of smooth muscle cells, on the abluminal surface of an endothelial cell in an ascending arteriole. Lumen (l) (× 9,600).

tudinally along the vessels, between the endothelial and smooth muscle cells or between the smooth muscle cells themselves (Fig. 10). These cords gradually became more sparse and finally disappeared at about the level of the superficial plexus. The elastic lamina comprised elastic fibre microfibrils surrounding and embedded within an amorphous material (elastin). Fine collagen fibrils ($46{\cdot}73 \pm 9{\cdot}03$ nm in diameter) were scattered amongst the elastic lamina.

The most distinctive and reliable feature distinguishing arterial from venous segments of the microvasculature was the structure of the basal lamina. In arterial vessels the basal lamina consisted of a single electron-dense band enveloping both endothelial cells and pericytes and which was sepa-

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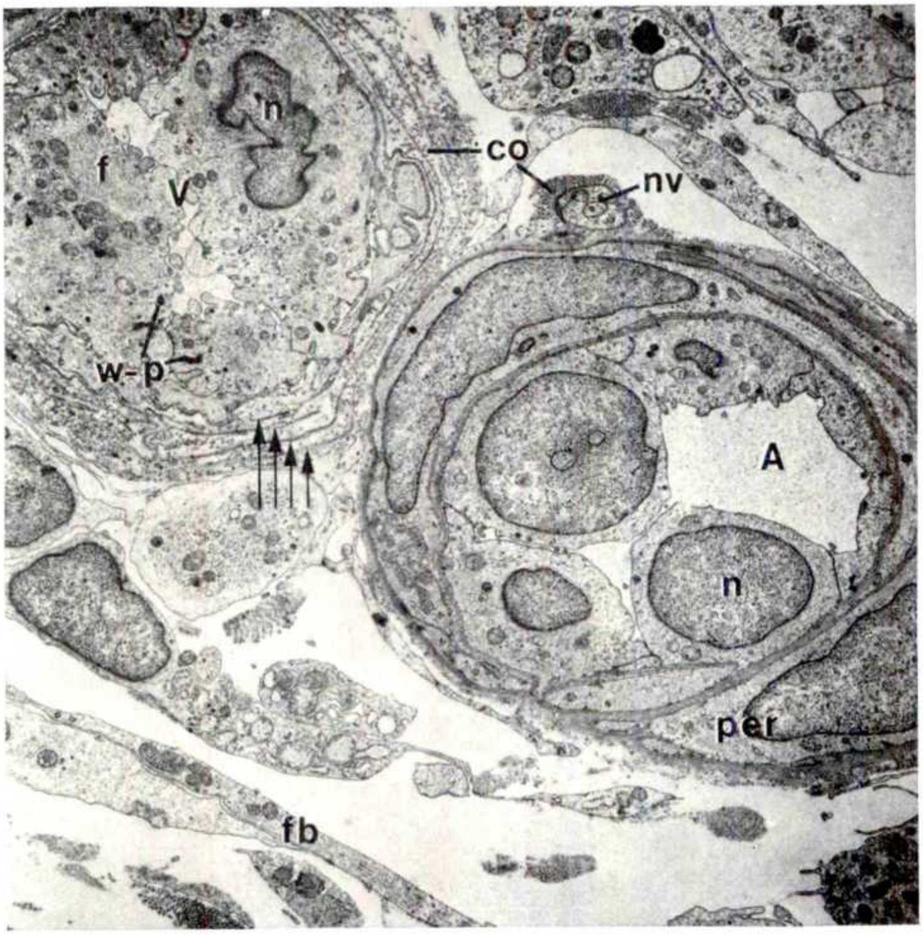
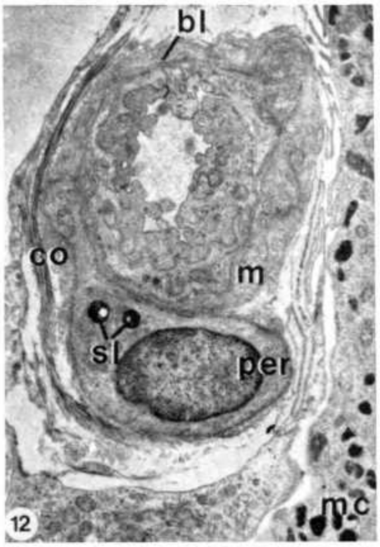
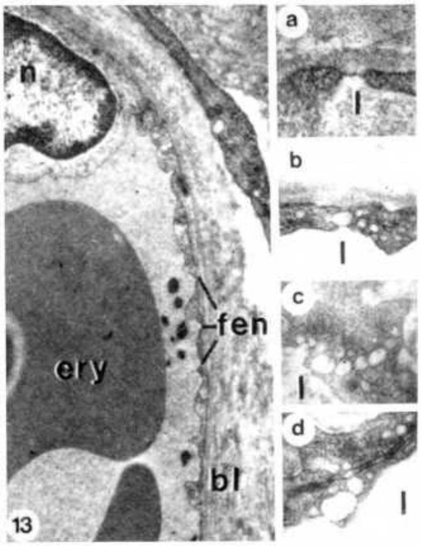


FIGURE 11. Electron micrograph of a cross-section through an arteriole (A) and a venule (V) in the superficial plexus. The arteriole possesses a relatively smooth-surfaced endothelium and a continuous pericyte layer (per). The endothelial nuclei (n) are rounded and regular in outline. A small unmyelinated nerve (nv) is closely associated with the vessel wall. By contrast, the venular endothelium exhibits irregular luminal and abluminal surfaces, an indented nucleus (n), many Weibel-Palade bodies (w-p) and abundant cytoplasmic filaments (f). Collagen (co) is interspersed among the multilaminated basal lamina (arrows). fb, fibroblast (× 5,300).

ated from these cells by an electron-lucent zone. The basal lamina appeared to merge with a homogeneous material of similar electron density which formed a continuous sheet around the vessel (Figs 10 and 11). In venous vessels (Figs 11 and 14) the basal lamina was multilaminated, comprising electron-dense bands ($67{\cdot}2 \pm 32{\cdot}72$ nm wide) separated by electron-lucent areas (30–500 nm wide). Collagen fibrils ($51{\cdot}58 \pm 11{\cdot}87$ nm in diameter) were interspersed between the laminations which were often discontinuous. At the tips of the capillary loops (Fig. 13) the basal lamina frequently displayed a few barely distinct laminations. Collagen fibrils ($69{\cdot}97 \pm 13{\cdot}07$ nm in diameter) surrounded vessels of all categories. Those associated with the basal lamina were significantly thinner ($P = 0{\cdot}001$; Student's '*t*'-test) than those found in the perivascular bundles.

With respect to the organelles of the smooth muscle cells and pericytes, mitochondria and ribosomes often occurred in groups near the nucleus, a feature that was more obvious in the smooth muscle cells.





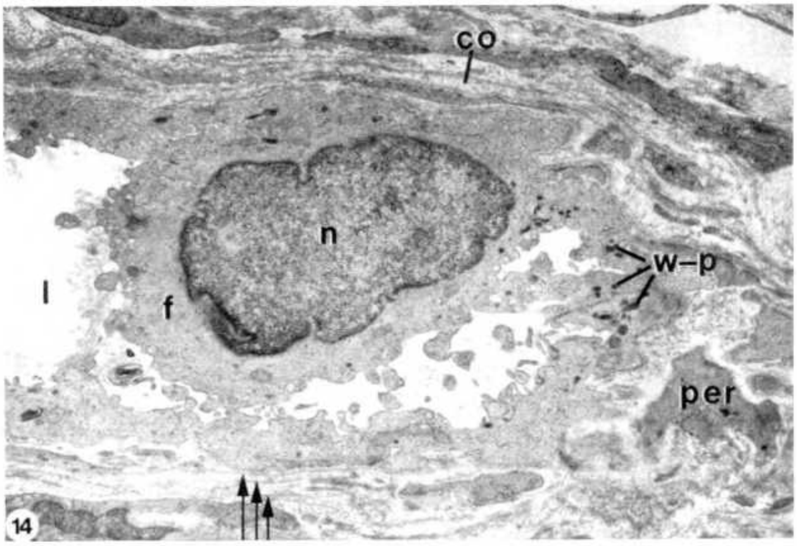


FIGURE 12. Electron micrograph of a transverse section through the arterial side of a capillary loop. The vessel is in a contracted or collapsed state. The pericyte layer (per) is discontinuous. The basal lamina (bl) appears as a single strand surrounding the endothelial cells and pericytes. A mast cell (mc) and fine collagen fibrils (co) are close to the vessel (× 4,400).

FIGURE 13. Electron micrograph of a section through the tip of a capillary loop adjacent to the overlying epidermis. Note the thin attenuated endothelium and several closed fenestrae (fen). The basal lamina (bl) displays weakly differentiated laminations. ery, erythrocyte in lumen; n, endothelial nucleus (× 7,700).

Inserts:

(a) Higher magnification of a closed fenestra. l, lumen (× 38,000).

(b) Detail of a vesicle fusing with both luminal and abluminal plasma membranes of an endothelial cell. l, lumen (× 16,700).

(c) A chain of vesicles, connected by only thin strands of membrane are stradling an endothelial cell. l, lumen (× 13,500).

(d) Two vesicles are fused to each other and form a channel from the luminal surface to lateral surface of an endothelial cell. l, lumen (× 16,700).

FIGURE 14. Electron micrograph of an oblique section through a postcapillary venule. Note the irregularly shaped endothelial cells, the indented endothelial nucleus (n) surrounded by bundles of cytoplasmic filaments (f), the abundant Weibel-Palade bodies (w-p) and multilaminated basal lamina (arrowed) interspersed with collagen fibrils (co). l, lumen; per, pericyte (× 5,800).

The muscle cells were characterized by the presence of fine (5–6 nm) filaments associated with dense plaques, often in contact with the cell membrane (Fig. 10). At the level of the superficial plexus these dense bodies became smaller, less distinct and less numerous. A few small dense plaques were also a feature of the pericytes. Surface-associated pinocytotic vesicles were numerous in the smooth muscle cells (Fig. 10) but sparser in pericytes especially along the cell surface adjacent to the endothelium (Figs 11 and 12). Distinct junctions between endothelial cells and pericytes or smooth muscle cells were not found.

Fibroblasts, mast cells and undifferentiated 'histiocytes' (Fig. 12) often surrounded vessels of all types. Nerves and blood vessels frequently followed a similar course and small unmyelinated nerve fibres were occasionally seen in close association with the outer layers of arterioles (Fig. 11). There was never any obvious contact or connection between nerves and vessels although sometimes the vascular basal lamina was contiguous with that of the nerve.

DISCUSSION

In the skin, the first obvious clue to a vessel's identification comes from its situation and relationship to other vessels. In this way Spalteholz (1893) was able to draw up a classification of human dermal blood vessels; a system which was later greatly simplified by Lewis (1927). Recently Mihm *et al.*, (1976) proposed a simple arrangement of normal human dermal microvasculature which is confirmed by our present investigation, although we have used some different terms for the various vascular segments. Yen & Braverman (1976) named the vessels in the superficial plexus which are invested with an elastic lamina and smooth muscle layer 'terminal arterioles'. We have reserved this term for those vessels without an elastic lamina but with a complete pericyte layer, which branch off the superficial plexus and connect directly with the capillary loops.

Owing to the large overlap between the diameters of the different vessel segments (Fig. 9) we do not consider a classification of vessel type based upon size is valid in our material. Measurements are most useful when a comparison can be made in the same section between two adjacent vessels, e.g. arteriole and venule in the superficial plexus. Measurements of vessel diameter in fixed tissue probably have only a limited bearing on function. Furthermore, differences due to fixation techniques render any comparison of results from different laboratories meaningless. Although this fact was recognized by Cowdry (1938) and Zweifach (1939), vessel diameters are still a regular feature of papers dealing with the morphology of microvasculature. Ideally, vessel nomenclature should be based upon identification of the physiological function of the vessel under question (Zweifach 1937). At present, this is best determined by relating morphological characteristics to possible function (Bennett, Luft & Hampton 1959; Karnovsky 1970) and considerable effort has been put into elucidating the functional significance of endothelial structure (see Majno & Joris 1978).

Ultrastructural features distinguishing endothelial cells of the venous system from those of the arterial side include their more irregular luminal and basal surfaces and their round and indented nuclei. This nuclear shape may reflect the ability of venular endothelium to undergo contraction (Majno, Shea & Leventhal, 1969): the projections on the plasma membrane providing a reservoir for subsequent relaxation. That arteriolar endothelium can also contract or change shape is possibly suggested by its containing dense plaques with associated actin-like filaments. To our knowledge this finding has not been recorded in normal dermal endothelium before although Eady and Wilson Jones (1977) found large numbers of dense plaques with associated fine filaments in the hypertrophic endothelial cells which characterize pseudopyogenic granuloma, an angioproliferative disorder of skin.

The occurrence in endothelial cells of coated vesicles, lysosomes, multivesicular bodies and myelin-figures, points to an endocytotic function comparable to that attributed to sinusoidal endothelium of bone marrow (De Bruyn, Michelson & Becker, 1975). This evidence is strengthened by the observation of acid phosphatase activity within dermal vascular endothelium (Higgins & Eady, 1981). Like Braverman & Yen (1977) we found that the number of pinocytotic vesicles opening onto the luminal surface of ascending arterioles was descreased compared to the rest of the microvasculature. Unlike Movat & Fernando (1964), however, we did not find that the vesicles were larger in venules.

We consistently found closed fenestrae at the tips of capillary loops on that side of the vessel facing the overlying epidermis. This feature was previously reported by Seifert & Klingmüller (1972), in many unspecified body regions. Takada & Hattori (1972) found fenestrated capillaries in finger and abdominal skin, and Pietra *et al.*, (1968) observed them in the heels of 2–5 h old infants. Fenestrae were also seen in capillaries in the perifollicular vascular plexus (McLeod, 1970). However, Braverman & Yen (1977) failed to find fenestrated capillaries in normal papillary dermis from the arms of several women.

Fenestrae are considered to be important in allowing rapid transfer of materials from the vessel into the surrounding connective tissue (Clementi & Palade, 1969; Simionescu, Simionescu & Palade, 1972) and possibly in the opposite direction (Casley-Smith, 1971). Their proximity to the epidermis, or intestinal epithelium (Clementi & Palade, 1969), is probably functionally significant and might support the concept of Ryan (1976) that blood vessels, epidermis and intervening connective tissue constitute an epidermal capillary unit in which blood vessel behaviour is influenced by the epithelium. Our observations of venous-type basal lamina investing fenestrated endothelium confirms Casley-Smith's (1971) earlier finding that fenestrae are more frequent at the venous than arterial end of the capillary, and therefore in the region thought to be the most permeable (Rouse, Gilding & Smith, 1930). The transendothelial channels that appeared to be formed from either a single pinocytotic vesicle (Fig. 13b) or several vesicles linking together (Fig. 13c and d) could also have a role in rapid transport of water and solutes across the vascular wall.

The structural difference between the basal lamina in the venous and arterial systems may reflect differences in the role of the basal lamina in control of blood vessel permeability, probably as a filter to plasma proteins. It may also indicate further functional variation between the endothelium and surrounding cells of arterial and venous vessels, since the basal lamina is synthesized by the cells it invests (Farquhar, 1978).

Weibel-Palade bodies were more frequent in the endothelial cells of the venular elements of the microvasculature. They were, however, never seen in the pericytes as reported by Zelickson (1966) in human skin capillaries. In none of the sections examined did we find a structure resembling a precapillary sphincter (Zweifach, 1939; Rhodin, 1967) or a postcapillary sphincter (Bosley, 1965).

Without the use of a tilting microscope stage critical examination of endothelial junctions was difficult although it was our impression that both tight junctions and gap junctions were present between endothelial cells of different vascular segments. By examining thin sections and freeze-fracture replicas, Simionescu, Simionescu & Palade (1975) have shown that the endothelial junctions in rat omentum and mesentery vary from one segment to another. It would be of great importance to know if this were also the case in human dermal vessels in view of the possible role of these junctions in blood vessel permeability (Wissig & Williams, 1978). Similarly, the elucidation of the functional significance of myoendothelial junctions, as found by Rhodin (1967) in rabbits and by Yen & Braverman (1976) in human dermal vessels, could be of equal importance. In the present study, however, no such structure was found.

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Skin lesions in angioimmunoblastic lymphadenopathy: histological and immunological studies

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SUMMARY

Angioimmunoblastic lymphadenopathy with dysproteinaemia is reported (AILD) in four patients with different skin pictures. As the disease progresses two main forms predominate: papulonodular and erythroderma. In all cases the histological picture of the skin mirrors that of the lymph-node.

Our results point to an increase in the peripheral blood, lymph-nodes and skin of T and subsequently of B lymphocytes suggesting that a proliferation of helper T cells and hence activation of the B-cell subpopulation may be involved in the pathogenesis of some cases of AILD.

Angioimmunoblastic lymphadenopathy with dysproteinaemia (AILD) is a recently defined entity with a clinical picture of lymphadenopathy, hepatosplenomegaly, rash, fever, weight loss and polyclonal gammopathy. Histologically, lymph-nodes display: (1) effacement of node architecture; (2) pleomorphic infiltrate composed of lymphocytes, plasma cells, immunoblasts and varying numbers of histiocytes and eosinophils; (3) marked proliferation of finely arborized vessels and endothelial cell hyperplasia with deposition of a PAS-positive, amorphous interstitial material (Frizzera, Moran & Rappaport, 1974, 1975; Abu-Zahra, McDonald & Horne, 1975; Lukes & Tindle, 1975; Flandrin, 1976).

Skin lesions are present in 40% of cases. They are described as an itchy generalized maculopapular rash (Seehafer *et al.*, 1980) frequently associated with the taking of drugs, and acral cryoglobulinaemic purpura (Wechsler & Stavrides, 1977). Infiltrated nodular skin lesions with no history of drug use have also been reported by Matloff & Neiman (1978) and Lessana-Leibowitch *et al.* (1978).

We are presenting the case reports of four patients in whom the pathological picture is the same but the skin signs differ.

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CASE REPORTS

Case 1

A 73-year-old woman with an unremarkable past history was admitted in April 1976 with generalized pruritus and large erythematous, infiltrated patches with distinct borders on the limbs. Papular lesions subsequently appeared on the trunk. The picture included generalized lymphadenopathy.

Apart from mild normochromic, normocytic anaemia and eosinophilia (12%), the laboratory findings were within normal limits.

Skin biopsy revealed a dense, pleomorphic middle dermal and subcutaneous infiltrate consisting of lymphocytes, large immunoblasts, plasma cells, and eosinophils. Marked proliferation of finely arborized vessels with hypertrophic and hyperplastic endothelium was observed (Fig. 5).

Biopsy of an axillary lymph-node showed only patchy involvement of capsular and pericapsular tissue by a pleomorphic cellular infiltrate consisting of small lymphocytes, plasma cells in various degrees of maturation, numerous and variously large immunoblasts with vesicular nuclei, prominent nucleoli and intensely pyroninophilic cytoplasm, and scattered eosinophilic granulocytes. Increased vascular proliferation with abundant intercellular, amorphous PAS-positive material was very evident.

Complete skin and node remission was obtained by treatment with prednisone (25 mg/day for 6 weeks). Three months later, pruritus returned, coupled with intense erythroderma, oedema of the lower limbs, generalized adenopathy, and hepatosplenomegaly. Absolute lymphocytosis (4100/mm^3) and eosinophilia were noted in the peripheral blood. A second regression was achieved with steroid therapy. In September 1976, papulonodular trunk lesions (Fig. 1) and erythroderma, adenopathy, hepatosplenomegaly, fever, and anaemia reappeared 10 days after suspension of the treatment. The bone marrow displayed eosinophilia, lymphocytosis, increased plasma cells, and numerous immunoblasts. Resumption of steroids led to the complete disappearance of the dermatosis and a reduction of adenopathy and hepatosplenomegaly. This lasted until February 1977, when there was a recurrence of erythroderma, skin nodules, generalized adenopathy and increased ESR (80 mm in 1 h). Polyclonal hypergammaglobulinaemia (IgG 3514 mg/100 ml; IgA 650 mg/100 ml) was demonstrated electro-

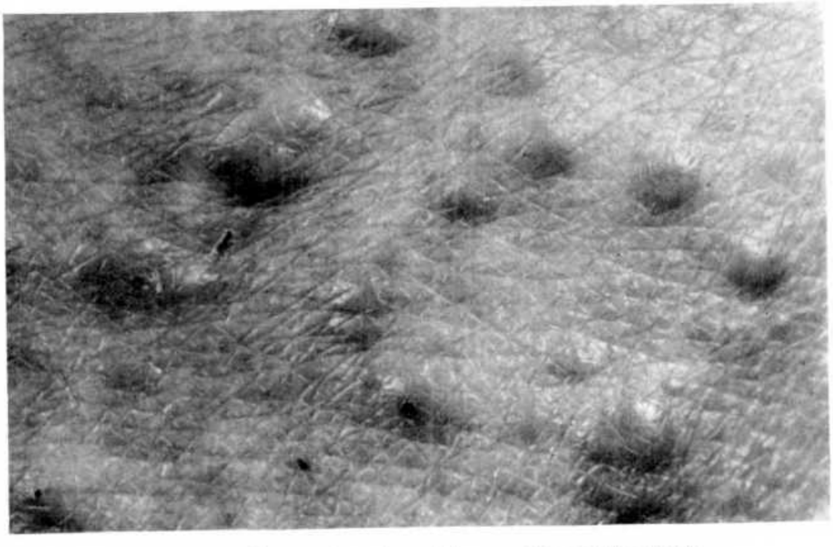

FIGURE 1. Case 1: papulo-nodular eruption on the trunk.

phoretically. Lymph-node biopsy revealed 'florid' AILD (Fig. 6). A steroid regimen was maintained until August 1977. During this time, the lymph-nodes shrank considerably, but never disappeared, though the spleen and liver could no longer be palpated. Large numbers of intensely pruriginous disseminated skin nodules then appeared (Fig. 2). These were the size of a pea but tended to grow to the size of a large nut. The surface was taut, smooth, yellowish, shiny, and marked by telangiectases (Fig. 3). IgA rose to 1148 mg/100 ml. The Coombs test was positive. Histologically, the skin picture mirrored the node findings with more pronounced vascular and immunoblastic components. A chest X-ray in October revealed large mediastinal masses and bilateral hilar adenopathy. The picture also included erythroderma, fever, general wasting, anaemia, leukopenia, oedema of the lower extremities and renal failure. A further lymph node biopsy showed numerous typical Reed-Sternberg cells. After three courses of systemic chemotherapy, she died of heart failure 21 months after the onset of the diease.

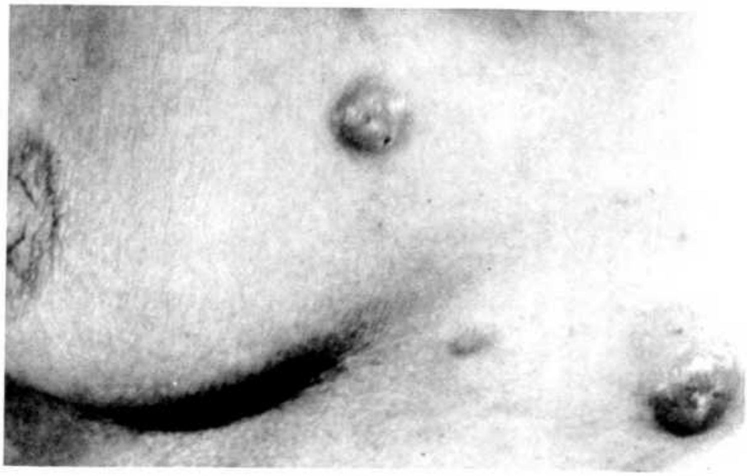

FIGURE 2. Case 1: nodular lesions.

Case 2

A 70-year-old man with no drug history displayed large erythematous patches that progressed to erythromelanoderma. Lymphadenopathy and hepatosplenomegaly were present. There were no constitutional symptoms. The laboratory results included high ESR (80 mm in 1 h), lymphocytosis without Sézary cells, eosinophilia, and polyclonal hypergammaglobulinaemia (IgG 4500 mg/% ml and IgA 1100 mg/100 ml). The bone marrow increase in eosinophils was slight (2·8%). Histological findings: in the dermis, chronic dermatitis with lymphocytes, numerous immunoblasts, histiocytes and scattered eosinophils and plasma cells. Sternberg-Reed cells were not observed. Lymph-node biopsy revealed the changes typical of 'florid' AILD.

Corticosteroid therapy diminished itching and the skin came less infiltrated, oedematous and erythematous. A decrease in node size was also noted.

Case 3

A 60-year-old woman with no significant past history had suffered from intermittent fever for 6 months. The symptoms included night sweats, malaise and weight loss. Three months later, a pruriginous papulo-macular eruption appeared (Fig. 4). This took the form of roundish lesions the size of a pea and proved resistant to steroid management. Generalized adenopathy was unaccompanied by

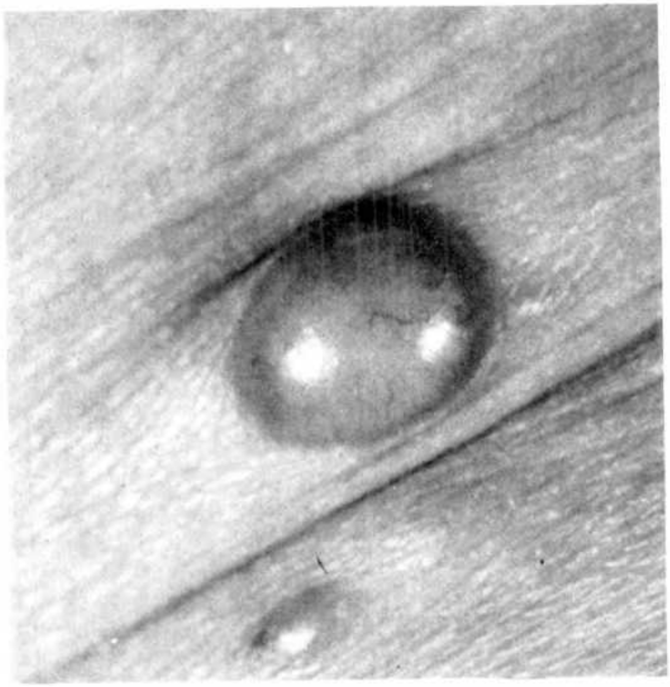

FIGURE 3. Case 1: large nodule covered by taut, smooth, yellowish skin. Note the numerous telangiectases.

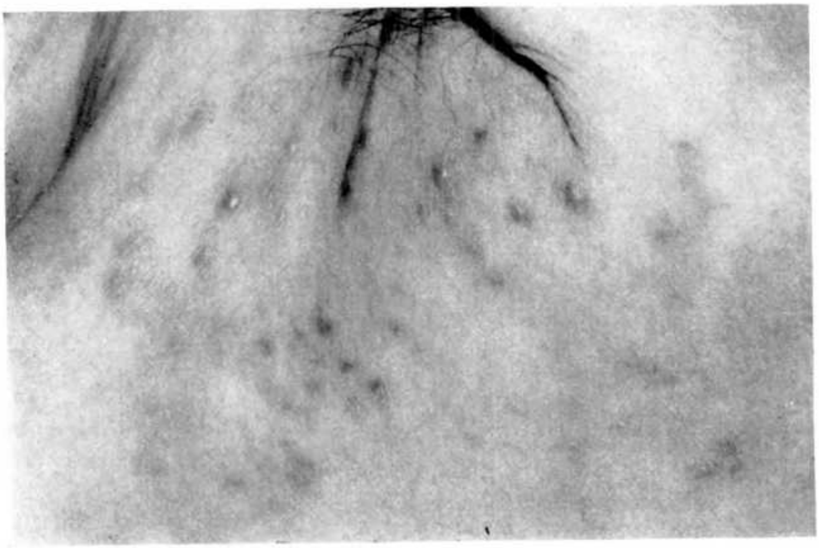

FIGURE 4. Case 3: generalized maculo-papular eruption.

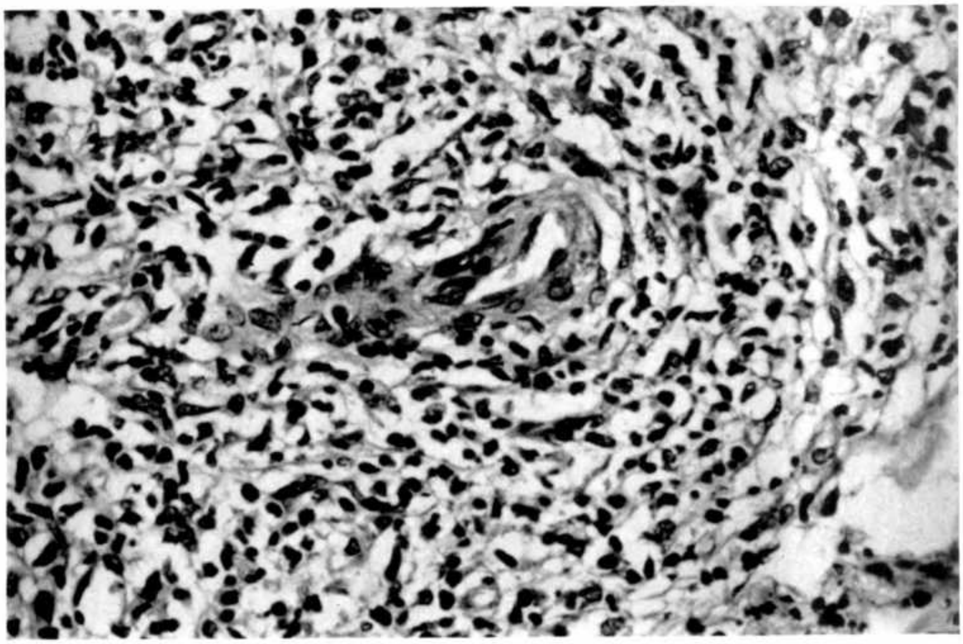

FIGURE 5. Case 1: skin biopsy. Dermal infiltrate consisting of lymphocytes, immunoblasts, plasma cells and eosinophils around a bood vessel with hyperplastic endothelium.

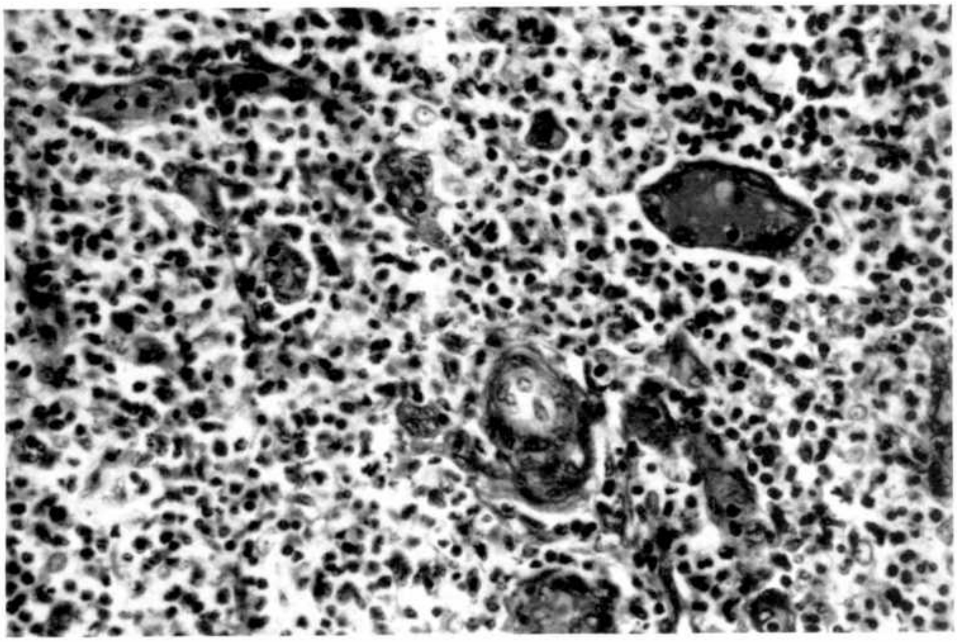

FIGURE 6. Case 1: lymph node biopsy. Mixed cell population and numerous vessels with thickened basement membranes and hyperplastic endothelium.

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hepatosplenomegaly. The laboratory findings included mild sideropenic anaemia, leukocytosis with eosinophilia (780/mm^3), increased ESR (92 mm in 1 h), and polyclonal gammopathy with IgG 3653 mg/100 ml. The cold agglutinin titre was over 1:16. Chest X-ray and tomography showed bilateral hilar and paratracheal adenopathy. Enlargement of the lumbar aortic nodes was also observed. Examination of the bone marrow revealed mild myelocytic and erythroid hyperplasia with increased eosinophil and plasma cell counts. Histologically the lymph-node and skin picture resembled that in case 1.

Steroids proved unsuccessful. The 'MOPP' regimen led to the disappearance of skin lesions and a decrease in node size. After the sixth course, erythematous, haemorrhagic papular elements appeared on the abdomen, with severe itching. No node enlargement took place, but the patient suffered from progressive wasting and died from sepsis 12 months after diagnosis.

Case 4

General pruritus without skin lesions appeared in August 1978 in a 71-year-old man who had been taking no drugs. In October, firm, painless, purple nodules appeared on the trunk and thighs, while the inguinal and cervical lymph-nodes became very large. The liver and spleen were not palpable. The patient reported a weight loss of 5–6 kg. The laboratory findings were normal.

TABLE 1. Distribution of T- and B-lymphocytes in peripheral blood, bone marrow, lymph node and skin in AILD patients

Case no.	Samples		% ERFC	% EAC	% SmIg				
					G	A	M	E	D
1	PB	4/76	65	19	14	1·5	0·5	1	0
		11/76*	57	22·5	27	4	22.5	5.5	4
		12/76*	42	30·5	38·5	4	32	3	13
		2/77	73.5	26	19·5	13	21	2	2
		10/77	46	33·5	16	1·5	26	4	8.5
		2/78	67·5	13	1	1·5	3	0	4·5
	BM		33	45	10·5	4·5	44	10	38·5
	L	4/76	71	2·5	2	0	2	0	0
		11/76*	46·5	21	—	—	—	—	—
		2/77	61	2·5	5	0	0·5	0	11
		2/78	70·5	5	0	2	1	0	0
	S		71	3	0	0	0	0	0
2	PB		63.5	27	5	0	1.5	1	3
	BM		44·5	35	6	—	1·5	2·5	6
	L		66	20	7	1	12	9	7
3	PB		63	18	2	0	14·5	0	3
4	PB		65	13	0·5	0	2	1	2·5

* During steroid therapy.
— Not done; PB = peripheral blood; BM = bone marrow; L = lymphnode; S = skin.

Histological examination of both the lymph-node biopsy and the skin showed the signs of AILD. After a 5-month period in which no symptoms appeared, new nodular skin lesions developed.

IMMUNOLOGICAL INVESTIGATIONS

E and EAC rosettes, were determined according to the methods described by Jondal, Holm & Wigzell (1972) and by Bianco, Patrick & Nussenzweig (1970) as previously reported (Bernengo *et al.*, 1977). B-cells bearing suface membrane immunoglobulins (SmIg) were detected by immunofluorescence staining according to the method of Pernis, Forni & Amante (1970) using monovalent fluorescein-conjugated rabbit antihuman immunoglobulin serum (anti-IgG, IgM, IgD, IgE, Kappa, Lambda, Cappel Lab.). Bone marrow, skin and lymph-node samples were processed as reported elsewhere (Bernengo *et al.*, 1979).

Peripheral blood, bone marrow, and lymph-node distribution of T and B lymphocytes was evaluated in cases 1 and 2. Investigations were confined to peripheral blood in cases 3 and 4. Immunological tests were carried out in the skin infiltrate only in case 1.

IMMUNOLOGICAL RESULTS

An increase in T cells was observed in skin, bone marrow and lymph-node (E-RFC percentage in bone marrow of seven controls with dermatopathic lymphadenopathy is $16{\cdot}2 \pm 5{\cdot}7$, in lymph-node $39 \pm 8{\cdot}2$). The immunoblasts were E-rosetting indicating their T-origin. T-cells decreased in the peripheral blood and lymph-node only during steroid management. A marked polyclonal increase in marrow IgM and IgD was present at the onset of the disease in case 1. In the peripheral blood of this case, T and B lymphocytes were determined monthly throughout the course of the disease. IgM and IgD receptors gradually increased along with IgG until the appearance of serum gammopathy (Table 1).

The lymphocytes from peripheral blood and from bone marrow were able to resynthesize, after trypsin digestion, surface IgM, IgD and IgE and, only partially, IgG. The same results were obtained after anti-Ig-induced redistribution (capping) of surface immunoglobulins.

DISCUSSION

Our four cases each offer a different aspect of AILD, even though the histological pictures in the skin and lymph-node were much the same and typical of the disease.

The taking of drugs could not be held responsible. The literature suggests that when AILD is triggered by a drug the skin signs are extremely varied at first: exanthematous rash (Frizzera *et al.*, 1975); purpura (Wechsler & Stavrides, 1977); generalized petechiae (Seehafer *et al.*, 1980); vasculitic, erythema multiforme and acute lupus erythematosus-like lesions (Lessana-Leibowitch *et al.*, 1978). As the disease progresses, however, two main forms predominate: large papulonodular lesions and erythroderma with the morphological picture and progression typical of lymphoproliferative disorders.

The skin and node histology in our four cases was exactly the same as that described in the literature. It is clear, therefore, that the skin, like the lymph-nodes, is one of the many organs that may be affected, perhaps earlier than the viscera, due to the homing ability of lymphoid cells.

AILD is not considered a malignant lymphoma. Spontaneous or steroid-induced remission and long survival are possible, provided serious intercurrent infection encouraged by cytotoxic treatments does not occur. Progression to immunoblastic sarcoma has, however, been reported by several authors (Lukes & Tindle, 1975; Howarth & Bird, 1976; Fisher *et al.*, 1976; Toth & Garam, 1977; Nathwani *et al.*, 1978).

Yataganas *et al.* (1977) observed a case that progressed to Hodgkin's disease, and the first of our cases also shows that this possibility exists.

Two pathogenetic mechanisms have been proposed: abnormal hyperimmune disorder of the B-cell system (Lukes & Tindle, 1975) and a graft versus host response mechanism (Graham, 1974; Frizzera *et al.*, 1975). A few reports, despite their incompleteness, have emphasized a T-cell deficiency in AILD (Iseman, Schwarz & Stanford, 1976; Palutke, Khilanani & Weise, 1976; Kosmidis *et al.*, 1978; Neiman *et al.* 1978).

Immunological investigation prior to any form of treatment in our patients on the contrary showed that T-cell values were increased in the peripheral blood, lymph-node, bone marrow and skin. In the skin lesions, T-cells predominated and the immunoblasts present were derived from a T-cell line, as in cutaneous T-cell lymphomas. In one case serial studies were performed throughout the course of the disease so we succeeded in proving that no impairment of T-cells occurred until the terminal phase. The increase in surface receptors suggests that an imbalance between T-helper and T-suppressor cells exists and that a proliferation of T-helper cells and hence the activation of the B-subpopulation may be involved in the pathogenesis of some cases of AILD.

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T Lymphocytes and mononuclear phagocytes in the skin infiltrate of systemic and discoid lupus erythematosus and Jessner's lymphocytic infiltrate

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SUMMARY

T Lymphocytes and mononuclear phagocytes were measured quantitatively with the histochemical acid α-naphthyl acetate esterase (ANAE) method from paraffin sections of skin affected by systemic and discoid lupus erythematosus and by Jessner's lymphocytic infiltrate. The composition of the patchy cutaneous mononuclear cell infiltrates was similar in these three disorders.

It has not been established whether discoid lupus erythematosus (DLE) and systemic lupus erythematosus (SLE) are different diseases or variations of the same disease. Certain clinical features, such as overlapping in histopathological, haematological and immunological features, the transformation of DLE to SLE, and skin lesions typical of SLE in DLE, favour some relationship between SLE and DLE (Rowell, 1979). Despite some differences in histological appearance, most authorities find it impossible to differentiate between SLE and DLE with ordinary histological methods on skin biopsy alone (Clark, Reed & Mihm, 1973).

This study was undertaken to see if differences exist in the frequency of T lymphocytes and mononuclear phagocytes in the skin lesions of SLE, DLE and Jessner's lymphocytic infiltrate.

MATERIALS AND METHODS

Patients

Skin biopsy samples were obtained both from skin lesions and from normal skin of five patients with SLE, four with DLE and two with Jessner's lymphocytic infiltrate.

Tissue processing

For the demonstration of T lymphocytes and mononuclear phagocytes, the tissue samples were

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processed as described in detail elsewhere (Ranki *et al.*, 1980). Briefly the tissues were fixed in Baker's formol calcium for 12 h at 4°C, washed in buffer, dehydrated in acetone and cleared in xylene, all at 4°C, before embedding in paraffin.

Histochemical demonstration of ANAE

Tissue specimens deparaffinized in xylene with subsequent acetone (10 min each at 4°C) were incubated in a medium consisting of 40 ml of 0·0067 mol/l phosphate buffer, pH 5·3, 2·4 ml of hexazotized pararosaniline and 10 mg of α-naphthyl acetate (Sigma Pharmaceuticals, St Louis, Mo., USA) in 0·4 ml of acetone. The mixture was adjusted to pH 5·8 using 1 mol/l NaOH, and incubated for 3 to 4 h. Counterstaining was performed in a 1% aqueous solution of toluidine blue; the sections were dehydrated in methanol and then acetone and cleared in xylene, 10 min each, and mounted (Ranki *et al.* 1980).

RESULTS

Routine haematoxylin and eosin staining revealed the typical morphological features of the respective diseases in all cases. Histochemical ANAE staining showed excellent morphology, with T lymphocytes displaying one or more distinct intracytoplasmic dots (T pattern, Fig. 1), and mononuclear phagocytes displaying a diffuse cytoplasmic staining (M pattern, Fig. 1).

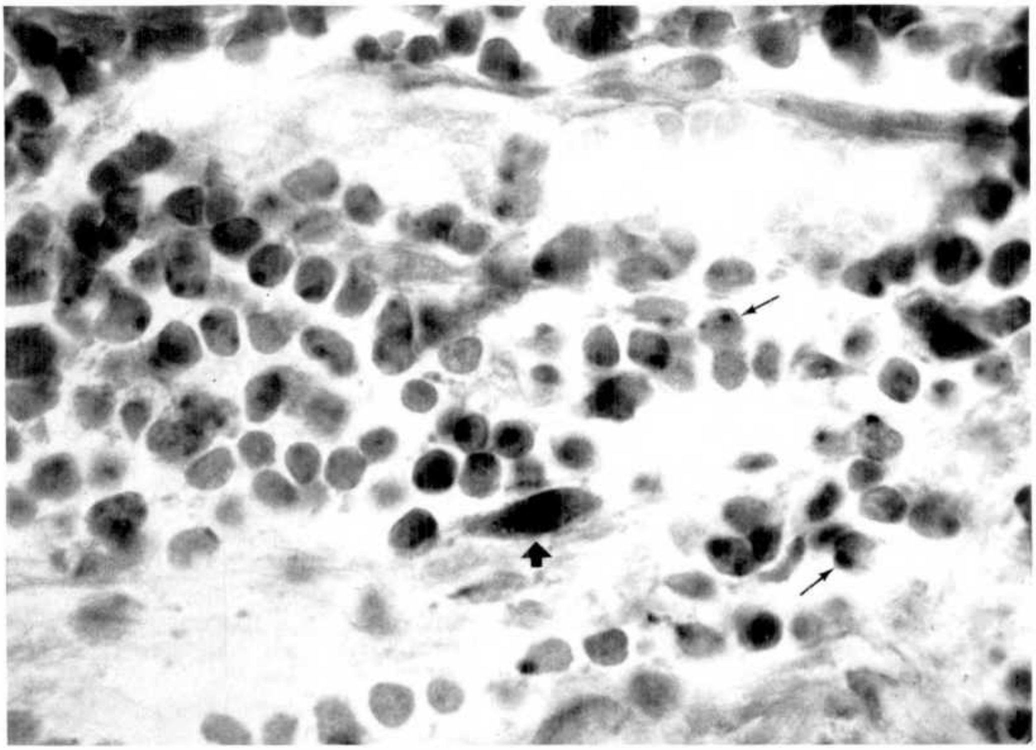

FIGURE 1. Cells of perivascular infiltrate displaying ANAE reactivity in SLE. T Lymphocytes showing one to several distinct dots in the cytoplasm (thin arrows); a mononuclear phagocyte showing diffuse cytoplasmic ANAE reaction (thick arrow) (× 1000).

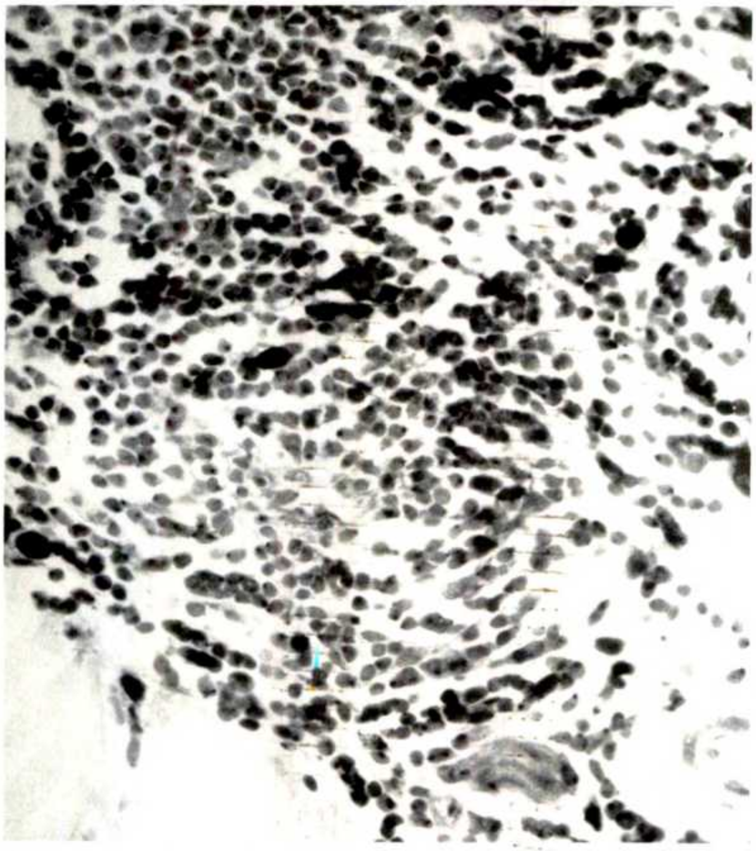

FIGURE 2. Perivascular infiltrate in DLE. A majority of the cells are T lymphocytes (× 1000).

No difference was seen in the compositions of the perivascular, periappendicular and subepidermal infiltrates (Figs 1–3). Table I shows that the majority of infiltrating lymphocytes were T lymphocytes, but both ANAE-negative lymphocytes and mononuclear phagocytes were also frequent. The proportion of different cell types in Jessner's lymphocytic infiltrate was the same as in normal peripheral blood. SLE, DLE and Jessner's lymphocytic infiltrates did not differ significantly from each other when compared using Student's t-test.

DISCUSSION

Patchy cutaneous mononuclear cell infiltrates present difficulties to the pathologist in diagnostic differentiation because of the difficulty in differentiating between T lymphocytes, B lymphocytes and mononuclear phagocytes. Application of the E rosette technique to frozen skin sections was unsuccessful (Edelson *et al.*, 1973) until Bjerke & Krogh (1978) introduced the combination of 2-aminoethylisothiouronium bromide hydrobromide (AET) treatment of sheep erythrocytes and the closed chamber technique. Antisera to human T lymphocytes have recently been applied to frozen skin sections to show that the majority of lymphocytes in DLE lesions are T lymphocytes (Chu, Fergin & MacDonald, 1979; Schmitt, Alario & Thivolet, 1979). These methods did not, however, permit the quantitative analysis of the T lymphocytes.

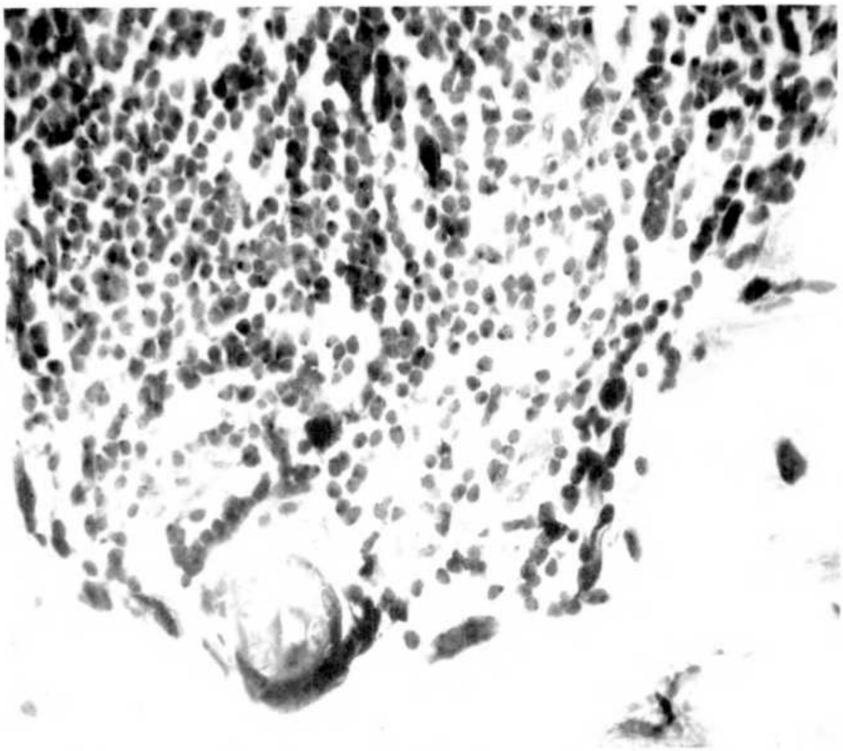

FIGURE 3. Inflammatory cells round a sweat gland in Jessner's lymphocytic infiltrate of the skin. A majority of the cells are T lymphocytes (× 400).

TABLE 1. Perivascular infiltrates in skin lesions as analysed with the ANAE method

Case No.	Diagnosis	Percentage of infiltrating perivascular cells			Total number of cells counted	Size of area counted (μm^2)
		T^A	M^B	Non-T Non-M		
1	SLE	62	19	19	96	7300
2	SLE	48	24	28	108	8000
3	SLE	62	4	34	370	16300
4	SLE	74	10	16	107	6600
5	SLE	35	9	56	123	6100
Mean ± s.d.		56 ± 15	11 ± 10	31 ± 16		
6	DLE	80	5	15	106	6700
7	DLE	64	16	20	140	8100
8	DLE	52	4	44	277	8800
9	DLE	79	5	16	292	15100
Mean ± s.d.		69 ± 13	8 ± 6	24 ± 14		
10	Jessner	85	6	9	235	8800
11	Jessner	71	2	27	598	24100
		78 ± 10	4 ± 3	18 ± 13		

T^Apattern = typical α-naphthyl acetate esterase staining pattern for T lymphocytes.
M^Bpattern = typical α-naphthyl acetate esterase staining pattern for mononuclear phagocytes.

The present results indicate that the majority of inflammatory cells in the dermis in SLE, DLE and Jessner's lymphocytic infiltrate contain acid α-naphthyl acetate esterase. This finding is compatible with the idea that they are T lymphocytes. Those cells which displayed no reactivity for ANAE are probably mainly B lymphocytes, null cells, or some of them may be activated T lymphocytes which have lost their ANAE activity (Tötterman, Ranki & Häyry, 1977). Similar proportions of T lymphocytes in SLE skin infiltrate have been shown in one case, using the ANAE method on frozen skin sections (Hovmark, 1977). The composition of Jessner's lymphocytic infiltrate resembles normal peripheral blood.

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Acid glycosidases in basal cell epithelioma and in seborrhoeic keratosis

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SUMMARY

Total activities of eleven glycosidases were determined in frozen-dried tissue fragments of basal cell epithelioma, seborrhoeic keratosis and epidermis. The values found were frequently much higher than those mentioned in the literature. Most enzyme activities were higher in basal cell tumours than in the adjacent epidermis; the differences between basal cell epithelioma and seborrhoeic keratosis were significant for only two of the eleven enzymes.

Intracellular glycosidases with pH optimum in the acid range are believed to be localized mainly in the lysosomes. The presence of these particles in skin has been supported by electron microscopic, histochemical, and biochemical evidence; the latter involved the determination of various hydrolases (Lazarus, Hatcher & Levine, 1975).

Lysosomes probably play a significant role in such diverse physiological functions of the skin as cellular division, keratinization and sebaceous secretion. There is increasing evidence that lysosomes also function in numerous pathophysiological circumstances. Such diverse processes as light sensitivity reactions, epidermal inflammation and neoplasia may be associated with lysosomal events. The lysosomal storage diseases, characterized by a primary defect in a lysosomal hydrolase, form a special and well-defined group.

Derivatives of 4-methylumbelliferone (4-MU) have been used for the measurement of hydrolase activities: the liberated 4-MU is strongly fluorescent and can be determined at very low levels, allowing the use not only of homogenates but also of very small tissue fragments (Hirsch, Duquette & Parks, 1976; Im & Hoopes, 1976; Robins, Hirsch & Emmons, 1968) or even of single cells (Hösli, 1977).

However, it has become apparent that if the lysosomal particles are left intact, total enzyme activity is not measured (Kolodny & Mumford, 1976) as the substrate does not penetrate through the membranes. Various methods have been used to disrupt the lysosomes and to release the active enzymes into solution: frozen sectioning and lyophilization, osmotic shock, sonication, freezing and thawing, pH changes, and/or detergent treatment.

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The 'latency' of the enzyme is defined as the percentage of the initially available ('free') activity as compared to the total activity after complete disruption of the particles (Touster, 1978).

But even when the particles are disrupted, some enzymes remain attached to the membranes and their activity is not assayed if the homogenate is centrifuged and only the supernatant is used. Moreover, there is evidence that factors other than binding to the lysosomal membrane are responsible for the association of acid hydrolases with sedimentable structures, e.g. nonspecific adsorption to various subcellular particles, binding to insoluble lysosomal matrix, entrapment within vesicles formed from nonlysosomal membranes and formation of insoluble complexes between enzymes and other macromolecules (Kint *et al.*, 1973; Patel & Koenig, 1976). In addition, one should remain aware of the heterogeneity of lysosomes and of the multiple forms of lysosomal enzymes, as well as of the possible presence of similar enzymes in soluble form and/or in other cellular structures (Barrett, 1972; Davies, 1975; Dean, 1975; Touster, 1978; Welman & Peters, 1976). It has also been found that the latency of the enzyme is not necessarily identical when different substrates are used for the assay.

Latency is different for each enzyme: β-glucosidase, β-acetyl-glucosaminidase, β-glucuronidase and β-xylosidase are more bound, while β-galactosidase and α-mannosidase are more easily soluble (Tappel, 1969). But latency also varies from tissue to tissue and from structure to structure in the same tissue (Patel & Koenig, 1976).

These problems are illustrated by the results of Steigerwald & Bartholomew (1973) on four glycosidases in human skin: the specific activities were three to six times higher in homogenates prepared from cryostat sections than in homogenates from tissue fragments. These authors postulated that sectioning at 6 μm intervals disrupts nearly all cells and facilitates enzyme release; they also noticed that their results were eight to ten times higher than those previously reported for the same enzymes in skin.

Several papers have been published with results on glycosidase activities in human and in animal skin (Clausen, Melchior & Paerregaard, 1972; Gerich, 1969; Gibbs & Griffin, 1970; Goldberg *et al.*, 1971; Levin, Fajerman & Jacoby 1972; Mier & van den Hurk, 1976; Öckerman, 1969; Ohkawara *et al.*, 1972; Steigerwald & Bartholomew, 1973). Comparisons, however, are difficult because some authors have used total skin and others more or less pure epidermis, because the method of extraction of the enzyme varies, because the substrates are not always the same, and because the activities are variously referred to wet weight, dry weight, or soluble protein.

In this paper we report on total activities of eleven glycosidases in human epidermal tumours (basal cell epithelioma and seborrhoeic keratosis) and in epidermis.

MATERIALS AND METHODS

Fifteen basal cell epitheliomas were used; each biopsy also contained adjacent normal epidermis in which the enzyme activities were also determined. There were eight seborrhoeic keratoses available; the activities were measured in the cellular tissue and in the overlying horny material.

The methods described by Lowry & Passonneau (1972) were used to prepare the skin samples: the biopsies are immediately frozen in solid carbon dioxide, cut in a cryostat to 30 μm sections and these are lyophilized. Small, histologically defined fragments of tissue are dissected under a binocular microscope and weighed on a quartz fibre balance. The average weight of these fragments is between 0·5 and 3 μg. They are placed in microtubes and used as an enzyme source. Three separate fragments were used for each enzyme. Such methods have been used with nervous tissue and with guinea-pig skin (Hirsch *et al.*, 1976; Im & Hoopes, 1976; Robins *et al.*, 1968).

The assays were done with 4-MU derivatives (Koch-Light, Colnbrook, England or Sigma, St

Louis, U.S.A.). The reaction mixture contained 1 mM substrate in 40 mM lactic acid-sodium lactate, the pH of which is given in Table 1. The volume of the mixture added to the tissue fragment was 10 μl.

After incubation (60 min at 37°C, except for β-acetylglucosaminidase and β-glucuronidase, for which incubation was only 30 min), 100 μl of carbonate-bicarbonate buffer, 0·2 M pH 10·5, is added, 100 μl is removed and diluted to 1100 μl with the same buffer in a fluorometer tube.

Fluorescence is measured in a Farrand Ratio fluorometer-2 against suitable quinine and 4-MU standards, with a Corning glass 7·37 as primary filter (maximal transmission at 360 nm) and Corning glasses 3·73 + 5·61 as secondary filter (maximal transmission 450 nm).

The results are expressed as millimoles of substrate hydrolyzed per hour incubation and per kg dry weight. The coefficient of variation of fifteen samples of the same biopsy was around 20% for each enzyme.

TABLE 1. Mean activities (as mmol of 4-MU substrates hydrolyzed per hour incubation per kg dry weight) and standard deviations (in parentheses) in basal cell epithelioma tumours (Y) (n = 15) and adjacent epidermis (E) and in seborrhoeic keratosis cells (SK) (n = 8) and horny material (H)

		Basal cell epithelioma		Seborrhoeic keratosis	
	pH	T	E	SK	H
α-Glucosidase	5·5	13·9 (8·4)	6·4 (3·3)	9·4 (5·8)	5·2 (3·4)
β-Glucosidase	4·6	9·1 (3·1)	8·6 (2·7)	9·1 (2·4)	5·8 (2·9)
α-Galactosidase	3·6	16·6 (9·3)	9·3 (4·0)	8·0 (2·3)	6·8 (3·3)
β-Galactosidase	3·6	44·5 (26·3)	20·3 (7·6)	25·2 (9·1)	20·1 (8·7)
α-Mannosidase	4·5	59·4 (30·7)	26·4 (8·5)	28·0 (8·5)	23·3 (10·2)
β-Acetylglucosaminidase	4·3	639 (281)	379 (92·6)	301 (103)	296 (134)
β-Glucuronidase	3·0	107·7 (94·2)	42·6 (31·2)	48·3 (28·9)	42·7 (22·9)
β-Xylosidase	4·0	0·54 (0·36)	0·51 (0·36)	0·67 (0·39)	0·40 (0·17)
β-D-Fucosidase	4·0	3·3 (1·54)	2·1 (0·80)	2·5 (0·61)	1·73 (0·50)
α-L-Fucosidase	4·0	51·0 (26·2)	23·6 (11·0)	30·4 (11·0)	22·1 (15·5)
α-Arabinosidase	4·0	3·2 (1·45)	1·92 (0·86)	2·76 (1·61)	1·71 (0·69)

No activity was found with 4MU-β-L-fucopyranoside as substrate.

RESULTS AND DISCUSSION

The mean values and standard deviations are given in Table 1.

Comparisons with values found in the literature can only be approximate for the reasons given above. Moreover, our basal cell epitheliomas were generally localized on the face of elderly patients and activities there might be different from those in the epidermis used by the other investigators. If one recalculates the measurements obtained in homogenates of normal epidermis by Mier & van den Hurk (1976) to our units, assuming that the epidermis is 70% water and 30% dry matter (Stüttgen & Schaefer, 1974), one finds values of 2·2 for α-glucosidase, 0·34 for β-glucosidase, 2·0 for α-galactosidase, 8·4 for β-galactosidase, 5·6 for α-mannosidase, 26 for β-acetylglucosaminidase and 10 for β-glucuronidase: these activities are lower than ours by a factor of between two and fourteen and we think that this is due to the methods of sample preparation. Similarly low values can be calculated from the data of the authors who have also used 4-MU derivatives as substrates (Goldberg *et al.*, 1971; Öckerman, 1969; Ohkawara *et al.*, 1972), except for some determinations of Öckerman (1969) that are in the same range as ours. We do believe that it is difficult to draw conclusions from such results, when only an unknown but sometimes small fraction of the total available activity is measured, a fraction that could be different in the normal and pathological samples.

The differences between basal cell epithelioma tumour tissue and epidermis are statistically significant ($P<0{\cdot}01$) for all enzymes except for β-glucosidase and β-xylosidase. The differences between seborrhoeic keratosis cells and horny material are significant for α-glucosidase and probably significant ($0{\cdot}01<P<0{\cdot}05$) for β-glucosidase and β-D-fucosidase. The differences between the mean activities in basal cell epithelioma and in seborrhoeic keratosis reach probable significance only for β-galactosidase and β-acetylglucosaminidase.

Obviously the enzymes present in the horny material of seborrhoeic keratosis have not been synthesized there, but are left-over proteins carried upwards during the keratinization process; apparently most enzymes survive this process and delay without much loss of activity, with the notable exception of α-glucosidase.

The specific activities of lysosomal enzymes are often greatly (up to four to five fold) elevated in solid tumours as compared with their tissue of origin (Poole, 1973). This conclusion is based on many studies in man and in animals. Increased glycosidase activities were also measured in virus-transformed cells (Bosmann, 1972). A correlation has been found between tumour invasiveness and increased enzyme activities. Moreover, the presence of a significant pool of active extracellular lysosomal enzymes would appear to favour the invasiveness of the tumour cells. The only moderate increase of enzyme activities we measured in basal cell epitheliomas might thus be in relation to the low invasive potentiality of these tumours.

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Follicular psoriasis

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SUMMARY

This is a report on ten patients with follicular psoriasis. Two distinct clinical types could be distinguished. An adult form, seen especially in women, presented with widespread psoriasis in which follicular lesions occurred on both thighs as part of the efflorescence. Follicular lesions were aggregated to form isolated asymmetrical plaques on the trunks of children with inactive psoriasis.

The histological findings in follicular psoriasis varied according to the age of the lesion. In the early lesion there was a marked dermal infiltrate in which the mast cells were prominent but the hair follicle appeared normal. Older lesions were found to have nucleated cells in the ostium of the follicle. These findings suggest that the hair follicle may be affected as part of psoriatic involvement of the integument.

Follicular psoriasis is rarely mentioned in early dermatological literature (McLeod, 1920) and even its existence has been denied (Bazex, Dupré & Christol, 1965). The following is a report of the clinical and histological findings in ten patients.

PATIENTS AND METHODS

Ten patients with follicular psoriasis took part in the study, three boys and one girl below the age of 10 years in whom psoriasis had been present for from 1 to 4 years, and six adults whose ages ranged from 18 to 69 years who had suffered from psoriasis over periods ranging from 18 months to 23 years. Five follicular lesions, including an early lesion less than 1 week old, were excised from four patients. The biopsy material was fixed in formalin, processed, embedded in paraffin wax, stained with haematoxylin and eosin and examined under the light microscope. One section of each was stained for mast cells with toluidine blue

RESULTS

Follicular psoriasis occurred in six adults and four children. The adult form was bilaterally distributed on the anterior, lateral and posterior aspects of the thighs as part of a widespread efflorescence (Fig. 1)

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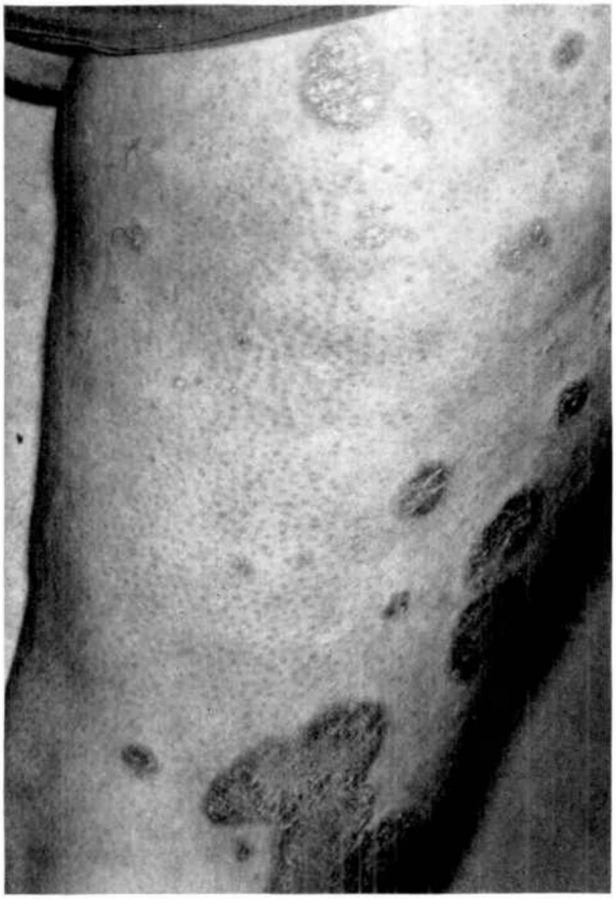

FIGURE 1. Follicular psoriasis of thighs showing variation in size of psoriatic lesions.

in five women and one man. Two patients (a male and a female) had been treated topically with clobetasol propionate and dithranol, respectively, just prior to the outbreak of the efflorescence. Aggregated follicular lesions formed asymmetrical plaques affecting the trunk and axillae of four children with inactive psoriasis.

Light microscopy of an early lesion showed follicular plugging with a perivascular and perifollicular distribution of an infiltrate consisting mainly of lymphocytes with an increase in mast cells demonstrated by staining with toluidine blue. Histology of older lesions (Fig. 2) showed follicular plugging with marked parakeratosis in the mid-zone of the ostium. The epidermis contiguous with the ostium was mildly hyperplastic with loss of the stratum granulosum.

DISCUSSION

Although the number of patients with follicular psoriasis is small, the findings were thought worth reporting because of the paucity of literature on this manifestation of the disease. Michelson (1958) described two forms of follicular psoriasis occurring in childhood: localized plaques comprising

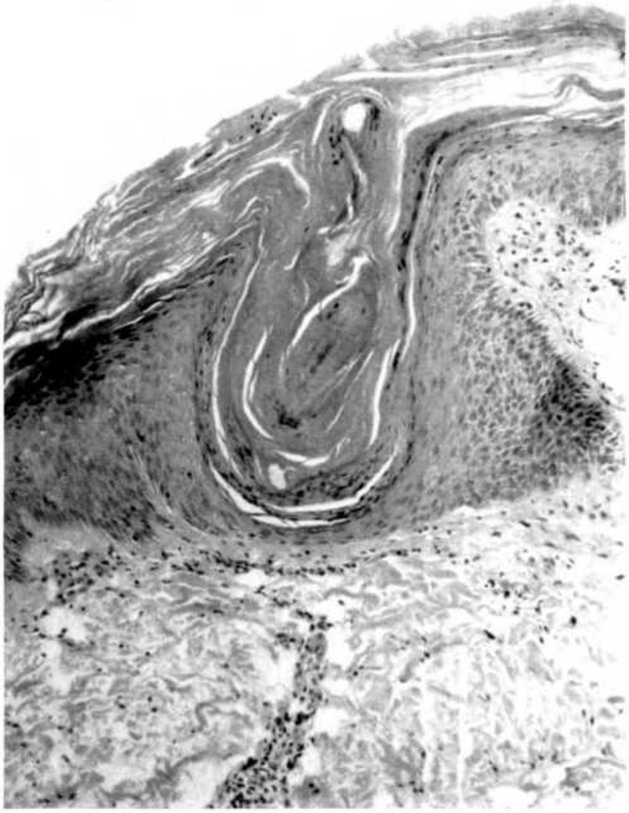

FIGURE 2. Histology of follicular lesion. There is parakeratosis in the ostium and the granular layer is absent in the epidermis neighbouring the ostium (H & E, × 140).

horny spikes found over bony prominences and a widespread form resembling pityriasis rubra pilaris. This study shows two clinical types of follicular psoriasis. An adult form affected mainly females in whom follicular lesions appeared around the thighs as part of a widespread efflorescence. A childhood form presented with asymmetrical plaques of aggregated follicular lesions in children with inactive psoriasis.

Histology of follicular lesions showed that dermal changes preceded follicular involvement. In early follicular lesions there was a dermal infiltrate with an increase in the number of mast cells. Braun-Falco (1971) has previously reported an increase in the number of macrophages and mast cells as the first significant cellular reaction during the development of a psoriatic lesion.

Initial investigations of Kúta & Neumann (1957) suggested an epidermal origin for psoriasis. A subsequent study (Neumann & Kúta, 1958) was carried out from which these workers concluded that psoriasis originates in the hair follicle. Ressa (1965) was not able to confirm their findings. He applied croton oil to hair follicles of fifty patients with active psoriasis. Only two patients developed psoriasis at sites of application, and histological examination in one showed a dermal origin at a site remote from the hair follicle. In the present study the first changes were in the dermis and support the observations of Ressa (1965) and the view that follicular changes are part of psoriatic involvement of the integument. Other supportive evidence for this view is the rarity of follicular psoriasis which often appears after classical clinical lesions, and the presence of psoriasis on palms and soles, sites devoid of hair follicles.

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Repeated patch testing in penicillin allergy

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SUMMARY

Fifteen patients with positive patch test reactions to penicillin preparations were retested several times. This resulted in increasing reactions in eight out of fifteen patients and in decreasing reactions in five patients.

Erythematous maculopapular eruptions sometimes develop during or after penicillin therapy. Whether these reactions are to be considered toxic or allergic (type IV) reactions is not always clear and so these patients have to be screened for the existence of a penicillin allergy. Fellner *et al.* (1969) demonstrated that the reactions to intracutaneous skin tests for delayed type hypersensitivity showed fluctuations. In order to see if we could detect similar changes, epicutaneous tests with penicillins (van Ketel, 1975) were performed repeatedly. Fifteen patients with positive patch test reactions to pencillin (tested about 4 weeks after the drug eruption had subsided) were retested, if possible, at 10, 16, 64 and 100 weeks after the clinical reaction. These patients had developed a rash while they received in six cases amoxicillin, in four fenethicillin, in two ampicillin, and for one patient the drug was unknown. Test preparations were: penicillin G 200,000 u/ml in water, almocillin (600,000 u procaine penicillin G and 2% aluminium monostearate) as is, bicillin (600,000 u procaine penicillin G and 200,000 u sodium penicillin G) as is, while ampicillin, amoxicillin, fenethicillin, cloxacillin and two cephalosporins (cefadrin and cefalotin) were tested as powder. The test preparations gave regularly negative results in controls. The results of the readings after 48 h are summarized in Figs 1 and 2. In eight out of fifteen patients the number of positive reactions per patient increased (Fig. 1a). All but one of these patients also showed increasingly severe reactions on the patch tests (Fig. 2a). In contrast to these results, five patients were found with a decreasing number of positive reactions and seven patients showed weaker reactions (Figs 1b and 2b).

These results differ from the observations of Fellner *et al.* (1969) who noticed only decreasing reactions. Their explanation for this effect is that it could have been caused by hyposensitization. Our increasing reactions may be due to the frequent patch testing, and/or the exposure to small amounts of penicillin in food acting as a booster for the sensitization. However it is also possible that these observations are nothing more than 'normal' physiological variations of the immune response of the individual patient towards penicillin.

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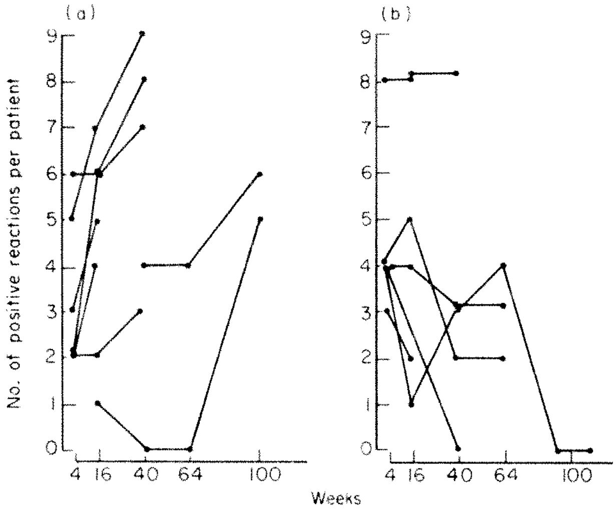

FIGURE 1. The numbers of positive reacting penicillin reactions are shown for each of the fifteen patients at subsequent patch testing, if possible at 4, 16, 40, 64 and 100 weeks after the clinical onset of the initial allergic manifestation. (a) Those showing an increase. (b) Those showing a decrease.

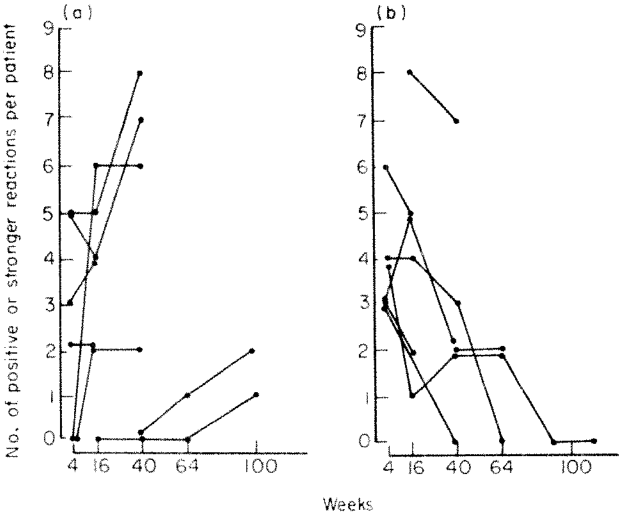

FIGURE 2. The total number of positive or stronger positive reactions which each individual patient showed at subsequent patch testing at the same times as in Fig. 1. (a) Those showing an increase. (b) Those showing a decrease.

van Ketel (1975) observed that epicutaneous testing with penicillin in patients with negative reactions to penicillin gave no evidence of active sensitization when repeated a year later. We therefore consider the overall risk of sensitization by patch testing negligible. But our investigations in patients with positive reactions suggest that repeated testing may result in stronger sensitization. Therefore it seems advisable to avoid frequent patch testing with penicillins.

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The influence of ultraviolet radiation on allergic contact dermatitis in the guinea-pig. I. UVB radiation

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SUMMARY

Guinea-pigs were sensitized by percutaneous application of dinitrochlorobenzene and exposed to UVB (280–320 nm) radiation. The exposure to radiation diminished the response to an elicitation dose of the hapten administered 14 days later within the site of irradiation. The exposure dose of radiation required to produce this effect resulted in a marked erythemal response, but this response did not conceal the contact allergic reaction. The site of elicitation of the allergic response had to be included in the exposure field.

The influence of exposure to non-ionizing radiation on allergic contact dermatitis has received little attention. In one study (Haniszko & Suskind, 1963), multiple exposures to UVB (middle-wave ultraviolet, 'sunburn' spectrum, 280–320 nm) radiation depressed cutaneous reactivity to 2,4 dinitrochlorobenzene (DNCB) in the guinea-pig. The effect was confined to irradiated skin and no attempt was made to determine whether the diminished response resulted from irradiation of the induction or the elicitation site. Reactivity to DNCB was also found to be transiently depressed in mice chronically exposed to UVB radiation (Jessup *et al.*, 1978). The depression in contact skin sensitivity observed in that study was not confined to exposed sites and was reversible, even while exposure to radiation was continued. Based on cell transfer experiments, it was suggested that exposure to UV radiation produced a block at the level of antigen recognition in the induction phase of the immune response. In these experiments DNCB was administered by injection rather than by percutaneous application. The aims of the present study were to re-examine the effect of UVB radiation on allergic contact dermatitis and to determine the relative effects of exposure to radiation on the induction and elicitation of the response.

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MATERIALS AND METHODS

Experiments were performed with Hartley strain, female, albino guinea-pigs weighing 500–600 g. All studies were performed on the dorsal thorax of the animals. Two adjacent 6 × 7 cm areas of skin were clipped and depilated (Neet, thioglycollic acid) on alternate days throughout the experiment. These two sites were exposed to UVB radiation; one site was used for induction and one for elicitation of allergic contact dermatitis to DNCB using a previously described method (Catalona *et al.*, 1972). The induction dose of DNCB (250 μg in 0·1 ml of acetone) was applied using a 1·5 cm diameter ring to prevent spread of the solution. The elicitation dose of DNCB (125 μg in 0·1 ml of acetone) was similarly applied 14 days later and 4–6 h after the last exposure to radiation. The responses to antigenic challenge were evaluated 24, 48 and 72 h later using a scale of: 0 = no reaction; 1+ = minimally perceptible erythema; 2+ = pink erythema; 3+ = red erythema; 4+ = intense erythema plus induration. In preliminary studies it was established that the doses of DNCB used for induction and elicitation were the minimum doses required to yield 3+ or 4+ responses 24 h after challenge in nearly all animals.

Guinea-pigs were exposed to non-ionizing radiation daily using a U-shaped bench radiator lined with 26 FS-40 Sunlamp bulbs (Westinghouse, Bloomfield, NJ). The average irradiance for the integrated 280–320 nm waveband, at the level of the animals' backs, was 820 mW/m^2 as measured by a cosine-corrected UV spectroradiometer system (IL 783, International Light, Inc., Newburyport, MA). The irradiance was uniform to within ±10% over the entire field of exposure. The erythemal response to radiation was recorded 24 h after the last exposure using a scale of 1+ = pink erythema; 2+ = red erythema; 3+ = intense red erythema with swelling. In preliminary studies it was established that an allergic response to DNCB could be evaluated in the presence of a marked UV-induced erythemal response.

The effects of two radiant exposure doses were examined. In the first study of sixteen animals the daily dose was 246 J/m^2. In the second study of forty animals, the daily exposure dose was 492 J/m^2. Groups of four (Study 1) or ten (Study 2) guinea-pigs received irradiation at either, both or neither site of induction and elicitation of allergic contact dermatitis (see Tables 1 and 2). Irradiation of the area which contained the site for induction was commenced 5 days prior to induction and continued

TABLE 1. Allergic contact dermatitis to DNCB in sensitized guinea-pigs exposed to UVB radiation and challenged on day 14. Response to the elicitation dose of DNCB read at 3 time intervals after application of chemical and evaluated on a 0 to 4+ scale. Four animals in each group. The dose of radiation (246 J/m^2 daily) produced a minimal erythema.

Group	Sites exposed*		Mean score of reaction to challenge dose at			Overall mean score
	Induction	Elicitation	24 h	48 h	72 h	
IE	X	X	4·0	3·5	2·5	3·3
I	X	—	4·0	3·0	3·0	3·3
E	—	X	4·0	4·0	2·8	3·6
O	—	—	4·0	3·8	2·8	3·5

*X = Site exposed; — = site not exposed.

TABLE 2. Allergic contact dermatitis to DNCB in sensitized guinea-pigs exposed to larger doses of UVB radiation and challenged on day 14. Response to the elicitation dose of DNCB read at 3 time intervals after application of chemical and evaluated on a 0 to 4+ scale. Ten animals in each group. The dose of radiation (492 J/m^2 daily) produced a marked erythema with swelling.

	Sites exposed*		Mean score of reaction to challenge dose at			Overall mean score
Group	Induction	Elicitation	24 h	48 h	72 h	(±s.e.mean)
IE	X	X	2·6	1·5	0·4	1·5 (0·22)
I	X	—	3·6	2·7	2·0	2·8 (0·16)
E	—	X	2·6	1·8	0·8	1·7 (0·28)
O	—	—	3·8	3·2	2·2	3·1 (0·21)

*X = Site exposed; — = site not exposed.

until the day of elicitation. Irradiation of the area containing the site for elicitation was commenced on the day of induction and continued until the day of elicitation.

The results were analysed by means of the Student t-test.

RESULTS

Study 1

Skin sites exposed daily to 246 J/m^2 of UVB radiation developed a 1+ erythemal response 24 h after the last dose of radiation. The responses to the elicitation dose of DNCB are recorded in Table 1. There were no significant differences ($P > 0·5$) between the cutaneous allergic responses among the four groups of animals.

Study 2

Sites exposed daily to 492 J/m^2 of UVB radiation developed a 3+ erythemal response 24 h after the last dose of radiation. The responses to the elicitation dose of DNCB are shown in Table 2. The overall mean scores in Groups IE and E, in which the sites of elicitation were exposed to radiation, are significantly lower ($P < 0·001$) than the scores in Groups I and O. The difference between the results in Groups I and O is not significant ($P > 0·1$).

DISCUSSION

This study demonstrated that exposure of guinea-pigs to certain doses of UVB radiation produced a reduction of allergic contact dermatitis to DNCB. In these experiments the effect appeared to be local and dependent on both the site of irradiation and the radiant exposure dose. Under the conditions tested, the site of elicitation of the response had to be included in the field of exposure. Exposure of the induction site alone did not reduce reactivity. Furthermore, inclusion of both the induction and the elicitation sites in the field of exposure did not significantly increase the reduction observed. The dose of radiation required to produce a reduced response to DNCB resulted in a marked erythemal response. A smaller dose of radiation, producing only a pink erythema, had no detectable effect on the reactivity to DNCB.

This study confirms the previous observation (Haniszko & Suskind, 1963) that exposure to UVB radiation can affect allergic contact dermatitis and extends those findings by the demonstration of a differential effect on induction and elicitation of the response. There are a number of mechanisms by which radiation might suppress the expression of allergic contact dermatitis. These possibilities include: (a) an alteration of carrier (skin) proteins or an interference in the binding of the hapten with the carrier protein; (b) functional changes in Langerhans cells preventing normal antigen 'presentation'; (c) interference with the normal influx of effector lymphoid cells or with the expression of their activities at the site of hapten application; (d) changes in the ability of blood vessels or other components of the skin to react to mediators of inflammation. Further studies are required to establish the mechanism(s) involved.

ACKNOWLEDGMENTS

The authors are grateful to Ms Dale Kristof, Ms Jane Meloni, Mr Nicholas DeCola, and Mr Howard Lawson, whose enthusiasm and attention to detail made this study possible. Dr Joel Krugler kindly performed the statistical analysis of the results.

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The influence of ultraviolet radiation on allergic contact dermatitis in the guinea-pig.

II. Psoralen/UVA radiation

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SUMMARY

Guinea-pigs were sensitized by percutaneous application of dinitrochlorobenzene and exposed to UVA (320–400 nm) radiation following systemic administration of methoxsalen. This treatment diminished the response to an elicitation dose of the hapten administered 14 days later within the site of irradiation. The site of elicitation of the allergic response had to be included in the field of exposure, and reduction of the allergic response was observed only when the exposure to radiation was commenced at the time of induction of contact allergy. Established contact skin sensitivity was not affected by radiation.

Exposure to UVA (320–400 nm) radiation following systemic administration of psoralen (commonly referred to by the acronym, PUVA) is effective in the treatment of a variety of skin diseases including psoriasis, eczema, vitiligo and mycosis fungoides. The effectiveness of this treatment has led to an examination of possible mechanisms by which it may produce its beneficial and adverse effects. One area of study has been the influence of PUVA on the immune system and this subject has recently been reviewed (Morison, Parrish & Epstein, 1979). It has been suggested that PUVA may affect contact allergy (Volden, Molin & Thomsen, 1978). Patients with mycosis fungoides who had established contact skin sensitivity to nitrogen mustard lost their sensitivity following a course of PUVA therapy; this observation was uncontrolled and the effect of PUVA on allergic contact dermatitis has not been studied in an experimental system. The aims of the present study were to investigate the effect of exposure to PUVA on allergic contact dermatitis in the guinea-pig and to attempt to determine the relative effects of such exposure on the induction and elicitation of the immune response.

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MATERIALS AND METHODS

Experiments were performed with Hartley strain, female, albino guinea-pigs weighing 500–600 g. All studies were confined to the dorsal thorax of the animals; treated sites were clipped and depilated (Neet, thioglycollic acid) on alternate days throughout the experiments. Allergic contact dermatitis was induced by application of 250 μg DNCB in 0·1 ml of acetone and elicited 14 days later by application of 125 μg DNCB in 0·1 ml of acetone. DNCB was applied within a 1·5 cm diameter metal ring to prevent spread of the allergen (Catalona *et al.*, 1972). The response to antigenic challenge was evaluated using a scale of 0 = no reaction, 1+ = minimally perceptible erythema, 2+ = pink erythema, 3+ = red erythema, and 4+ = intense erythema with induration. The induction and elicitation doses of DNCB were the minimum doses found to give 3+ or 4+ responses 24 h after challenge in nearly all animals.

PUVA treatments were given daily and exposure doses were the same in both phases of the study. Methoxsalen (12 mg/kg body weight) was administered by gavage and 1 h later the animals were exposed to 27×10^3 J/m^2 of UVA radiation in a U-shaped bench radiator lined with 26 PUVA fluorescent bulbs (Sylvania, Danvers, MA). The average irradiance for the integrated 320–400 nm waveband at the level of the animals' backs was 45 W/m^2 as measured by a cosine-corrected UV spectroradiometer system (IL 783, International Light Inc., Newburyport, MA). The irradiance was uniform to within ±10% over the field of exposure. The erythemal response to radiation was evaluated 24 h after the last exposure using a scale of 1+ = pink erythema, 2+ = red erythema, and 3+ = intense erythema with swelling. In preliminary studies it was established that an allergic response to DNCB could be evaluated in the presence of a marked UV-induced erythemal response. Two studies were conducted.

Study 1

Forty-five guinea-pigs were divided into five groups. Induction and elicitation doses of DNCB were applied within two adjacent 6×7 cm skin areas on the dorsal thorax of the animals. Treatment of these two areas varied as outlined in Table 1. Irradiation of the areas containing the site for induction was commenced 5 days before induction and was continued until the day of elicitation. Irradiation of the areas containing the site for elicitation was commenced on the day of induction and continued until the day of elicitation; the elicitation dose of DNCB was applied 4 h after the last exposure to radiation. The responses to the elicitation doses were evaluated after 24, 48 and 72 h.

TABLE 1. Allergic contact dermatitis in sensitized guinea-pigs exposed to various combinations of methoxsalen/UVA radiation, and challenged on day 14. Responses to the elicitation dose of DNCB read at three time intervals after application of chemical and evaluated on a 0 to 4+ scale. The radiant exposure dose (27×10^3 J/m^2 daily) produced a marked erythema and swelling

Group	No. of animals	Psoralen	Sites irradiated*		Mean score of reaction to challenge dose at			Overall mean score (±s.e.mean)
			Induction	Elicitation	24 h	48 h	72 h	
IE	10	Yes	X	X	1·9	1·8	0·9	1·5 (0·28)
I	10	Yes	X	—	3·3	2·7	2·1	2·7 (0·22)
E	10	Yes	—	X	1·8	1·5	0 9	1·4 (0·28)
O	10	No	—	—	3·6	2·9	2·3	2·9 (0·26)
P	5	Yes	—	—	3·0	2·6	2·6	2·7 (0·32)

*X = Site exposed; — = site not exposed.

Study 2

Contact skin sensitivity to DNCB was induced in fifteen animals and their sensitivity was confirmed after application of an elicitation dose of allergen 14 days later. The animals were then divided into three groups and studied for a 14-day period as follows: five animals received psoralen and subsequent exposure of a 6×7 cm area of the back to UVA radiation daily for the 14 days; five animals received no treatment for the first 7 days and then received psoralen followed by exposure of a similar area to UVA radiation daily for the last 7 days of the 14 day period; five animals received no psoralen or UVA radiation. On the 14th day, an elicitation dose of DNCB was applied within the treated area and the response was evaluated 24 h later.

The results were analysed by means of the Student t-test.

RESULTS

Study 1

All sites exposed to UVA radiation following ingestion of psoralen developed a 3+ erythemal response as a result of the exposures. The responses to the elicitation dose of DNCB are given in Table 1. The mean scores in both Groups IE and E, in which the site of elicitation was exposed to radiation, are significantly lower ($P < 0.001$) than the scores in Groups I, O and P.

Study 2

The five animals that received fourteen PUVA treatments developed a 3+ erythemal response as a result of the cumulative exposures. The five animals which received only seven PUVA treatments developed a 2+ erythemal response from the exposures. The responses to the pre-treatment and post-treatment challenges with DNCB were evaluated as 4+ or 3+ in all animals and the results are shown in Table 2. The mean scores in each group were not significantly different ($P > 0.5$) either before or after treatment with PUVA.

TABLE 2. Allergic contact dermatitis in sensitized guinea-pigs exposed to psoralen/UVA radiation after establishment of sensitization to DNCB and prior to second challenge. Response to elicitation doses of DNCB prior to exposure to PUVA and after 0, 7 or 14 days of PUVA treatment. Response evaluated on scale of 0 to 4+

No. of animals	Mean pre-PUVA response to DNCB	Duration of PUVA treatment prior to challenge (days)	Mean post-PUVA response to DNCB
5	3·4	14	3·6
5	3·8	7	3·6
5	3·6	0	3·4

DISCUSSION

Under certain conditions, exposure of guinea-pigs to UVA radiation following systemic administration of psoralens produced a reduction of allergic contact dermatitis to DNCB. This effect was observed only in experiments in which exposure to PUVA was commenced at the time of, or before, induction of contact skin sensitivity. Exposure to PUVA, even in markedly erythemogenic doses, did not affect the elicitation of allergic contact dermatitis in animals with established sensitivity to DNCB.

Furthermore, in order to demonstrate inhibition of contact dermatitis, the site of elicitation of allergy had to be included in the area exposed to UVA radiation. Inclusion of the site of induction of contact skin sensitivity within the field of exposure did not increase the reduction of contact dermatitis, and exposure of the induction site alone did not produce a significant reduction.

Several interpretations of these findings may be considered. The effect of PUVA may be localized and exerted only upon the immune response generated at the site of elicitation. In that case it would be necessary to postulate that, in contrast to newly sensitized animals, animals with established contact skin sensitivity to DNCB are able to mount a quantitatively greater immune response, or qualitatively different immune response, at the elicitation site. A stronger immune response in guinea-pigs with established sensitivity to DNCB may overwhelm any detectable suppressive effect of PUVA. Alternatively, the different sensitivities of the primary and secondary immune responses to PUVA treatment may be due to differences in the mechanisms of these two responses. For example, Langerhans cells, which are located in the epidermis and therefore likely to be affected by exposure to radiation, may be more essential to the primary response as compared to the secondary response. Another explanation for the findings would be that, while PUVA does produce a local effect at the challenge site, it also has a systemic effect on the induction of contact allergy. Although such a systemic effect was not found in the present study, in which the maximum area exposed to radiation was 84 cm^2, preliminary studies in which the entire back of the animals (approximately 150 cm^2) was exposed indicate that, in addition to the local suppression at the elecitation site, PUVA also produces systemic suppression of contact allergy.

This study has shown that exposure to PUVA can affect an immune response *in vivo*. There is evidence that some of the skin diseases that respond to PUVA treatment, for example vitiligo and atopic eczema, have a pathogenesis that involves abnormal immune responses. It is therefore possible that the effectiveness of PUVA treatment in such conditions is due to a suppression or alteration of such abnormal immune responses in humans.

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Isolation and biochemical characterization of tyrosinase-rich GERL and coated vesicle in melanin synthesizing cells

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SUMMARY

The site of condensation and activation of tyrosinase prior to melanosome melanogenesis has been investigated using subcellular fractionation and enzymic analysis of so-called Golgi apparatus of melanoma cells. Two different functional units have been found in the so-called Golgi fraction, the tyrosinase-rich GERL-coated vesicle unit and the TPPase-rich Golgi unit. The tyrosinase-rich unit consists of a tubular structure with a cisternal portion corresponding to the three-dimensional structural characteristics of GERL, and spheroid vesicles, corresponding to the currently accepted criteria for coated vesicles. This fact suggests, in agreement with electron microscopic observations, that the GERL-coated vesicle unit is a specialized portion of smooth endoplasmic reticulum having the function of activating and condensing the tyrosinase synthesized by ribosomes.

Three-dimensional electron microscopic cytochemistry of active tyrosinase sites in melanin synthesizing cells has revealed that the deposition of the product of the dopa reaction is localized not in the sheet structured Golgi apparatus, but in the tubular GERL (Golgi apparatus-endoplasmic reticulum-lysosome system) and spheroidal coated vesicles. This was shown using high voltage electron microscopy with thick sections and a goniometer (Mishima, Imokawa & Ogura, 1979). These results led us to isolate the tyrosinase-rich tubular GERL and spheroidal coated vesicles from the so-called Golgi fraction and characterize them by biochemical and electron microscopic subcellular analysis. This paper is concerned with the three-dimensional structure and the specialized function of GERL.

MATERIALS AND METHODS

Separation of tyrosinase-rich compartments from the so-called Golgi fraction

Isolation of the so-called Golgi apparatus was performed by Seiji's method (Seiji, Moro & Tomita, 1978; Mishima *et al.*, 1979) using Greene's melanotic melanoma originally obtained from H.S.N. Greene and maintained in a Syrian (golden) hamster by successive subcutaneous transplantation.

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After suspension in a final concentration of about 1·2 M sucrose, the Golgi fraction (G_2) was incubated with the same volume of 1·0 or 10 mM dopa solution containing 0·05 M Tris-maleate buffer (pH6·9) for 60, 90, 100 or 180 min at 37 °C. The incubated mixture inducing melanization during the dopa reaction (Ishikawa, Fitzpatrick & Szabo, 1966; Hamada & Mishima, 1972) was layered immediately onto 1·2 M sucrose in a v/v ratio to sample of 2:3, and then centrifuged for 60 min at 105,000 *g* (SW rotor) to separate the so-called Golgi fraction (G_2) into tyrosinase-rich compartments as a sediment (dP_3) and tyrosinase-poor compartments as a non-sedimentable fraction (dG_3) remaining as a band floating between 0·5 M and 1·2 M sucrose. As a control, incubation and centrifugation with non-dopa containing mixture was conducted as before to obtain a non-sedimentable control fraction (G_3). The dG_3 and G_3-subfractions, collected from a band at the 1·2 M sucrose-homogenate interface with a Pasteur pipette, and the dP_3 particulate subfraction were further diluted three-fold with Tris-maleate buffer or 0·5 M sucrose, and then centrifuged for 60 min at 105,000 *g* to obtain each particulate fraction containing no remaining dopa.

Enzymatic assay

UDP-galactose N-acetylglucosamine galactosyl transferase activity was assayed by the method of Bergeron *et al.*, (1973) using 10 μl each of 0·3 M sodium cacodylate (pH 6·5), 0·3 M $MnCl_2$, 15 mM uridine diphospho-(^{14}C) galactose, 2·43 × 10^6 d.p.m./μmol 0·3 M N-acetylglucosamine, 0·5% Triton X-100 and the cell fraction containing about 40–60 μg protein. Glucose-6-phosphatase (G-6-Pase) was assayed by the method of Swanson (1955) using 60 mM maleic acid-NaOH buffer (pH 6·5) and 20 mM glucose-6-phosphate; 5′-nucleotidase (AMPase) by the method of Heppel & Hilmoe (1955) using 100 mM glycine-NaOH buffer (pH 8·5), 8 mM $MgCl_2$ and 2·4 mM adenosine-5-phosphate; thiamine pyrophosphatase (TPPase) by the method of Yamazaki & Hayashi (1968) using 33 mM sodium barbital (pH 8·0), 15 mM $CaCl_2$ and 3.3 mM cocarboxylase. Inorganic phosphorus was determined by the method of Chen, Toribara & Warner (1956) for G-6-Pase and AMPase, and by the modified method of Yamazaki & Hayashi (1968) for TPPase. Acid phosphatase (AcPase) was determined by the method of Shibko & Tappel (1965) using 5 mM p-nitrophenyl phosphate and 0·1 M acetate buffer (pH 5·0); tyrosinase by the method of Hamada & Mishima (1972) using 0·1 M phosphate buffer (ph 6·8) and 1 mM dopa. Protein was determined by the procedure of Lowry, Rowebrough & Randall (1951).

Electron microscopy

Isolated fractions were observed electron microscopically by the technique of negative staining using a 1% aqueous solution of phosphotungstic acid (PTA) (Cunningham *et al.*, 1974). High resolution scanning electron microscopy of the fractions was carried out by field emission scanning electron microscope JOEL JFSM-30 after fixing with glutaraldehyde and then osmic acid, drying by the critical point method and metal coating with gold-palladium.

Labilizing experiments

Subcellular fractions of 1·0 ml were incubated at 0–4 °C for 60 min with 1·0 ml of either 0·2% Triton X-100 aqueous solution (pH 7·0) or distilled water (pH 7·0) as a control. After incubation, this mixture was centrifuged at 20,000 *g* for 10 min and the extent of labilization evaluated by measuring the activity of enzymes released into the supernatant and those remaining in the sediment. Enzymes assayed were tyrosinase and AcPase.

RESULTS

Morphology of Golgi fraction

The so-called Golgi fraction isolated subcellularly by the current methods (Seiji *et al.*, 1978; Morre *et al.*, 1970) was analyzed on the basis of its characteristic morphology by electron microscopy using PTA negative staining. Electron microscopy of the so-called Golgi fraction has revealed the heterogeneity of its organelles. Figure 1 shows the Golgi apparatus-rich portion of the fraction. Golgi apparatus is characterized by the sheet structure with polygonal compartments divided by multiple fenestrations continuous with the plate-like central regions (Fleischer, 1969, 1979; Ehrenreich *et al.*, 1973). The GERL-rich portion of the so-called Golgi fraction is shown in Figure 2. GERL is composed of the three-dimensionally tubular, anastomosing tubular and cisternal portions (Novikoff *et al.*, 1971; Hand & Oliver, 1977; Boutry & Novikoff, 1975). Further the three-dimensionally tubular structure of GERL budding coated vesicles at their tip can be seen. Figure 3 shows the contained numerous coated vesicles which appear as small spheroid bodies having a coated surface structure and diameter of 40–100 nm. Three-dimensional electron microscopic observation using a high resolution field emission type scanning microscope has correspondingly revealed the tubular structure with budding tip of GERL in the so-called Golgi fraction (Fig. 4). Thus, the current method of subcellular isolation for so-called Golgi apparatus is found to include not only Golgi apparatus but also GERL and coated vesicles.

Enzymatic properties of the so-called Golgi fraction

As Table 1 shows, the so-called Golgi fraction contains a high activity of TPPase and galactosyl transferase, marker enzymes of the Golgi apparatus, and still has tyrosinase activity. Furthermore, G-6-Pase and AMPase, marker enzymes for rough endoplasmic reticulum and plasma membrane, are found to be low in the range of 10–30 nmol inorganic phosphate released/min/mg protein. In accordance with

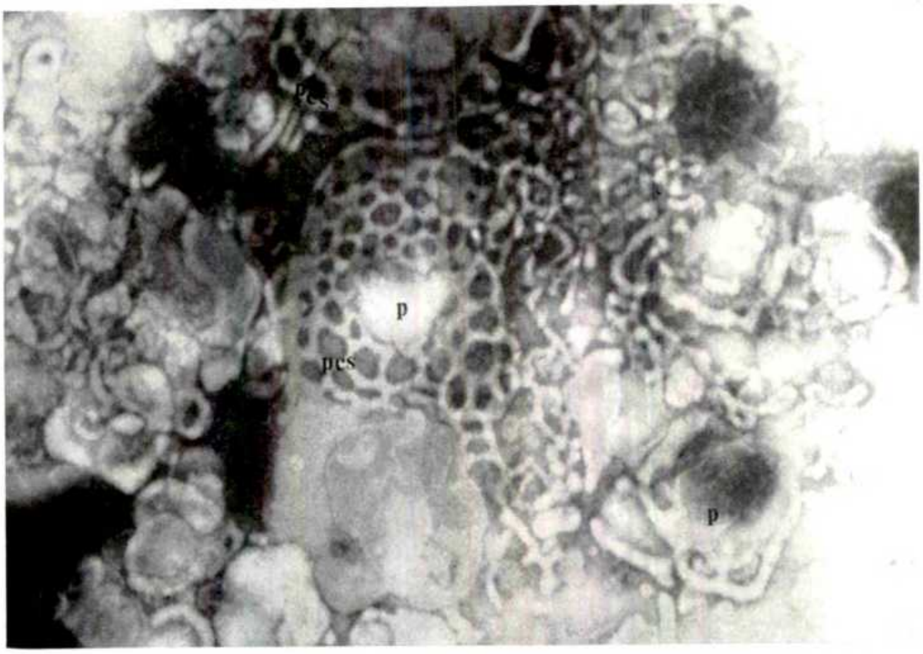

FIGURE 1. Golgi apparatus-rich portion of the so-called Golgi fraction isolated subcellularly from Greene's hamster melanotic melanoma. Golgi apparatus is characterized by the sheet structure having polygonal compartment (pcs) divided by multiple fenestrations continuous with the plate-like central regions (p). Negative stain with 1% phosphotungstic acid (pH 7·0) JEM-100S (80 KV) (× 64,900).

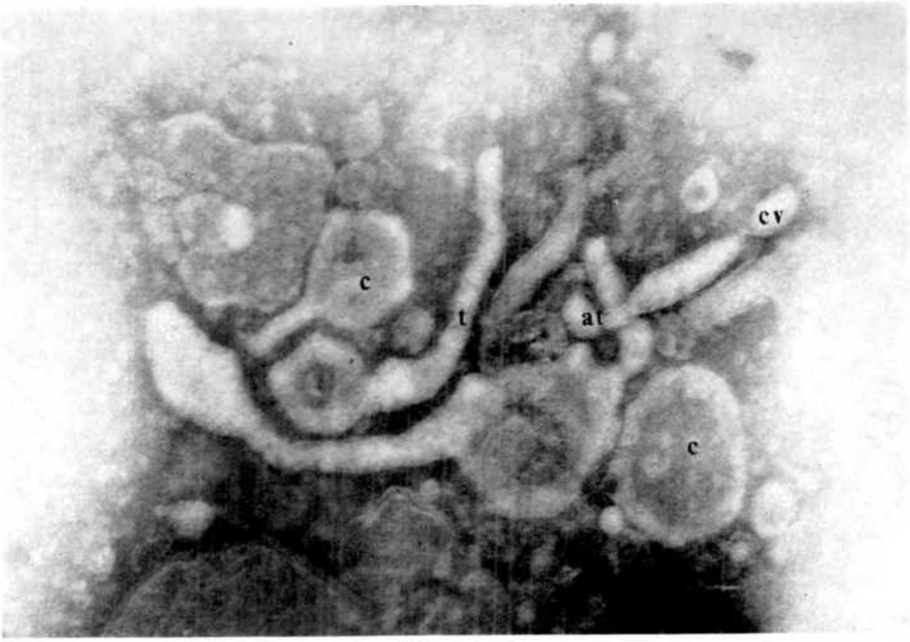

FIGURE 2. GERL-rich portion exhibiting cisternal portion (cs), tubular (t) and anastomosing tubular (at), from the tip of which coated vesicle (cv) is budding off. Negative stain with 1% phosphotungstic acid (pH 7·0) JEM-100S (80 KV) (× 86,450).

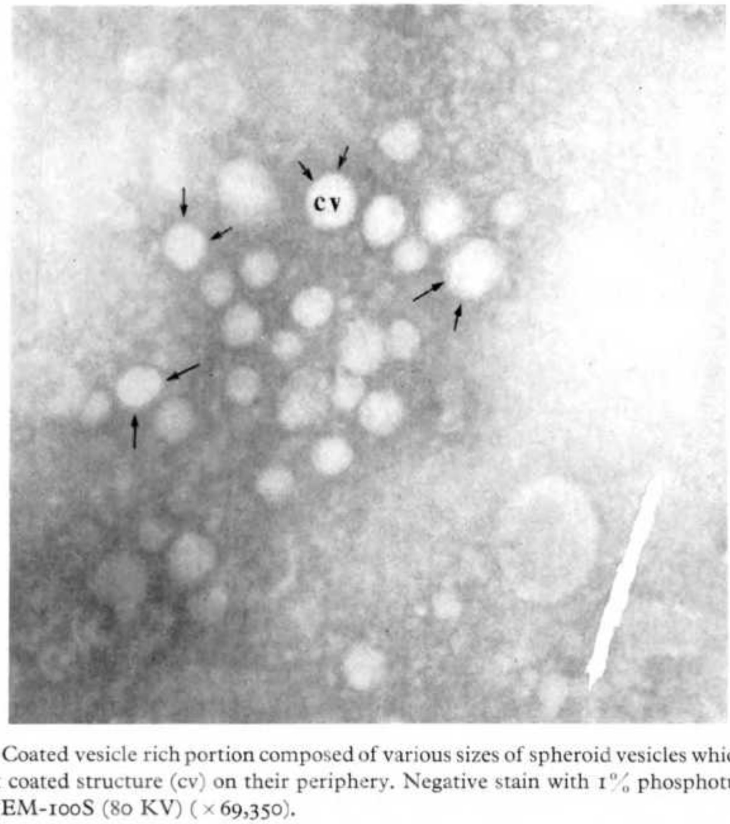

FIGURE 3. Coated vesicle rich portion composed of various sizes of spheroid vesicles which occasionally exhibit coated structure (cv) on their periphery. Negative stain with 1% phosphotungstic acid (pH 7·0). JEM-100S (80 KV) (× 69,350).

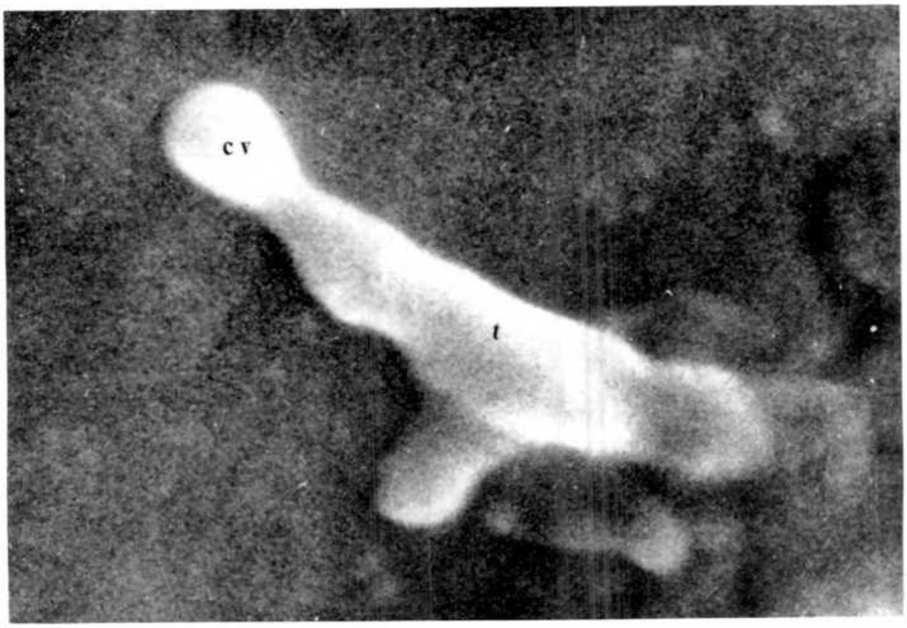

FIGURE 4. High resolution field emission type scanning micrograph of tubular GERL structure (t) budding off coated vesicle (cv) at the tip. GTA-OsO_4-Gold-Palladium coating. JSM-F7 (× 155,400)

TABLE 1. Enzymatic properties of isolated so-called Golgi fraction

Enzymes	Homogenate	Golgi fraction
Galactosyl transferase (nmol transferred UDP-galactose/h/mg protein)	6·6	114·9
5′-Nucleotidase (AMPase) (nmol released Pi/min/mg protein)	6·6	29·0
Glucose-6-phosphatase (G-6-pase) (nmol released Pi/min/mg protein	2·9	10·5
Tyrosinase (ΔE_{475}/10 min/mg protein	0·03	0·27
Thiamine pyrophosphatase (TPPase) (nmol released Pi/min/mg protein)	21·5	226·1
Acid phosphatase (AcPase) (nmol released p-nitrophenol/min/mg protein)	32·6	90·1

the above electron microscopic observation with PTA negative staining, the biochemical analysis of the isolated Golgi fraction suggests that there are essentially different functional units in the fraction.

Isolation and characterization of tyrosinase-rich compartments in the Golgi fraction

The precise separation of the tyrosinase-rich compartments from the so-called Golgi fraction is found to be difficult using only sucrose-gradient ultracentrifugation, but could be easily obtained after dopa preincubation (Hamada & Mishima, 1972; Ishikawa *et al.*, 1966). As shown in Table 2, it is possible to divide the so-called Golgi fraction into tyrosinase-rich sedimentable particles (dP_3) and tyrosinase-

TABLE 2. Tyrosinase and thiamine pyrophosphatase in fractions isolated by differential ultracentrifugation after preincubation using 1mM dopa for 100 min at 37 °C. In these conditions the highest tyrosinase activity was found in the dP_3 subfraction together with minimum TPPase activity. Experiments were carried out in triplicate

	Tyrosinase ΔE/10 min/mg protein	TPPase Pi released/min/mg protein
G_2	0·194 ± 0·045	128·3 ± 5·2
dG_3	0·150 ± 0·050	107·4 ± 10·5
dP_3	0·441 ± 0·035	16·7 ± 2.5

poor non-sedimentable (dG_3) subfractions. The dP_3 subfraction thus obtained exhibited very high levels of tyrosinase activity in the range of 0·4–0·5 E_{475}/10 min/mg protein, as compared to the non-sedimentable (dG_3) subfraction remaining as a band floating between 0·5 M and 1·2 M sucrose concentrations. It is also found that no or minimum activity of TPPase (Golgi marker enzyme) is present in the dP_3 subfraction.

Concerning the effect of dopa-preincubation on tyrosinase, the activity of the dP_3 subfraction is found to be maximum at 1 mM dopa preincubation for 100 min under conditions in which no substantial loss of TPPase is observed in the dG_3 subfraction. Although loss of tyrosinase activity due to melanization of these organelles is seen after 180 min preincubation with 10 mM dopa, substantial amounts of tyrosinase activity remain after 100 min preincubation with 1 mM dopa.

Membrane stability of organelles in the dP_3 subfraction has been examined by the release of tyrosinase and AcPase after Triton X-100 treatments in comparison with other subfractions associated with Golgi apparatus, G_2 and dG_3. As shown in Table 3, the substantial release of tyrosinase into 20,000 *g* × 10 min supernatant is seen to occur only in the dP_3 subfraction after 60 min preincubation

TABLE 3. Release of tyrosinase and acid phosphatase by Triton X-100 from tyrosinase-rich dP_3 particulate subfraction into 20,000 *g* × 10 min supernatant (Sup), following 60 min incubation at 4 °C

			Acid phosphatase* (% release)		Tyrosinase† (% release)	
G_4	Triton X-100	Sup	115·0	(296·4)	0·575	(87·8)
		Pet	44·8	(48·5)	0·069	(37·5)
	Control	Sup	38·8		0·655	
		Pet	92·3		0·184	
dG_3	Triton X-100	Sup	68·4	(198·3)	0·329	(80·4)
		Pet	43·1	(73·5)	0·119	(103·5)
	Control	Sup	34·5		0·409	
		Pet	58·6		0·115	
dP_3	Triton X-100	Sup	50·0	(793·7)	1·346	(246·1)
		Pet	13·4	(60·4)	0·179	(59·5)
	Control	Sup	6·3		0·547	
		Pet	22·2		0·301	

* nmole released p-nitrophenol/min/mg protein

† ΔE_{475}/10 min/mg protein. Pet: pellet after 20,000 *g* × 10 min centrifugation.

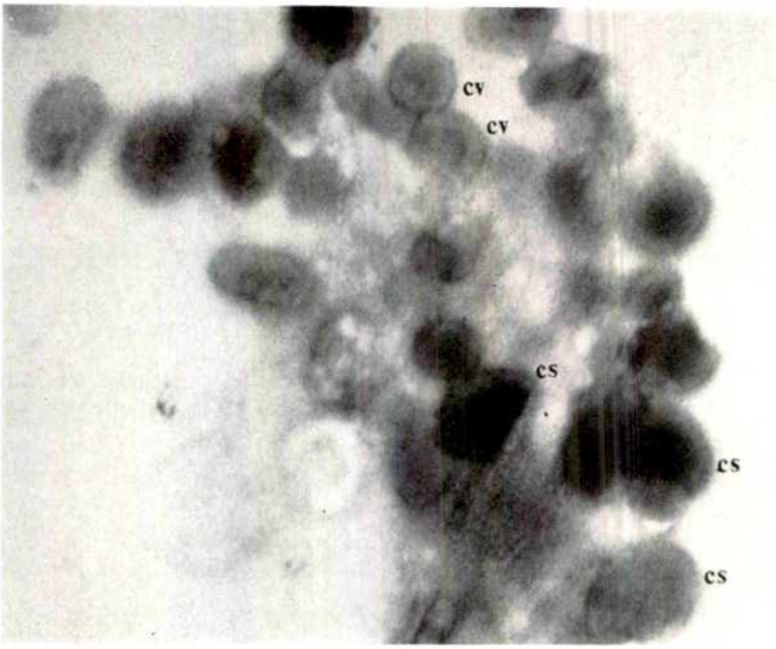

FIGURE 5. Tyrosinase-rich sedimentable subfraction (dP_3) exhibiting cisternal-like structures (cs) 220 nm in diameter, and coated vesicle (cv) 100–120 nm in diameter. Many structures have dopa melanin deposition. Negative stain with 1% PTA (pH 7·0) JEM-100S (80 kV) (× 60,350)

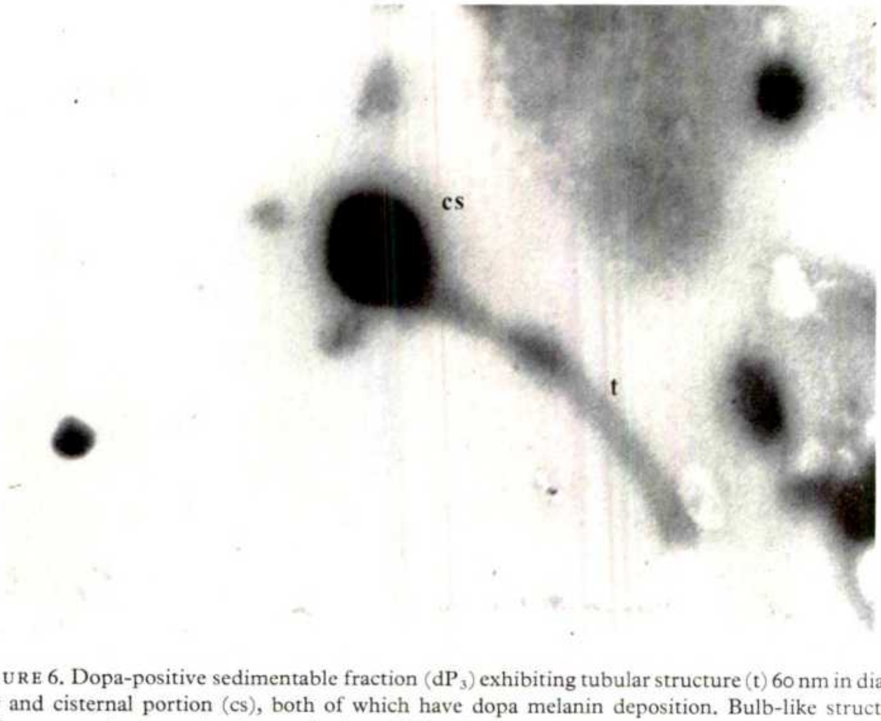

FIGURE 6. Dopa-positive sedimentable fraction (dP_3) exhibiting tubular structure (t) 60 nm in diameter and cisternal portion (cs), both of which have dopa melanin deposition. Bulb-like structure having strong dopa reaction is seen in the middle of the tubular structure. Negative stain with 1% PTA (pH 7·0) JEM-100S (80 kv) (× 38,350).

with 0·2% Triton X-100 at 0–4 °C. On the other hand, the release of AcPase is observed in all the subfractions.

Electron microscopy of the dP_3 particulate subfraction using PTA negative staining reveals mainly spheroid vesicles and the cisternal-like structures without tubules deposited with dopa melanin (Fig. 5). Cisternal portion with tubular structure, which corresponds to the three-dimensional structure of GERL, is occasionally seen having dopa melanin deposition (Fig. 6). However, neither sheet structured Golgi apparatus nor its broken fragments are seen in the dP_3 particulate subfraction.

DISCUSSION

GERL was originally described in neurons by Holtzman, Novikoff & Villaverde (1967), Novikoff *et al.*, (1971) and Novikoff (1973) as a specialized portion of smooth endoplasmic reticulum having the function of activating and condensating lysosomal enzymes and of forming lysosomes. In pigment cells, GERL's function and structure have been discussed in relation to the intracellular distribution of the specific melanin synthesizing enzyme tyrosinase. Novikoff, Abbala & Biempica (1968) first demonstrated, using the B-16 and Harding-Passey melanomas, the GERL as the initial tyrosinase-positive site which is clearly differentiated from the tyrosinase-negative Golgi apparatus by the electron microscopic dopa reaction. Similar intracellular localization of tyrosinase in GERL was observed by Maul & Brumbaugh (1971) in the regenerating fowl feather and by Hunter, Mattaz & Zelickson (1970) in human epidermal melanocytes irradiated by UV. However, most other investigators still consider such an initial active tyrosinase site to be part of the Golgi apparatus with a distinct polarity. Since these different theories were based principally on thin section electron microscopic studies using the dopa reaction, an additional approach is required for more distinct clarification of the structural and functional characteristics of GERL in pigment cells.

If the tyrosinase-rich compartment within the Golgi area has the same three-dimensional structure as the tyrosinase-negative structure which is composed of multi-layered sheets with polygonal window-like fenestrations, one may reasonably consider this tyrosinase-rich compartment as a part of the Golgi apparatus.

Recently it has been found (Mishima & Ogura, 1974; Mishima *et al.*, 1979), by using a high voltage electron microscope with a goniometer and thick sections of dopa-reacted B-16 melanoma, that the reaction product of dopa is localized not only in the coated vesicles and in a number of the premelanosomes, but also three-dimensionally in the tubular, anastomosing and cisternal portions of the GERL. This dopa-positive structure is distinctly different from Golgi lamellae which have a thiamine pyrophosphatase reaction positive, multi-layered sheet structure. These findings led us to investigate further the tyrosinase-rich smooth membrane system after its subcellular separation followed by biochemical and non-sectioning electron microscopic analysis. Using a purified fraction of the tyrosinase-rich smooth membrane subcompartment (1·8–3·4-fold increase in tyrosinase compared to G_2) obtained by differential ultracentrifugation after dopa preincubation, a substantial decrease of the Golgi marker enzyme TPPase (7–10-fold decrease compared to G_2; see Table 2) and elimination of the multi-layered sheet structure characteristic of Golgi apparatus were found. Compared with the decrease of TPPase the relatively less marked increase in tyrosinase in the dP_3 subfraction may be explained by the supposition that, under the conditions selected for dopa preincubation, the dG_3 fraction still contains some portion of tyrosinase-containing compartments. On the other hand, dP_3 contains no or much less contamination with Golgi apparatus. Our electron micrograph of the dP_3 subfraction confirms the presence of the three-dimensionally tubular structures of GERL continuous with its cisternal portion and the induction of melanization occurring in them although most of GERL seems to lose its structural integrity during the isolation process. The biochemical and structural properties of the tyrosinase-rich smooth membrane subcompartment isolated by our present dopa preincubation differential ultracentrifugal method is found to coincide with the currently accepted criteria for GERL rather than for part of the Golgi apparatus structure.

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Pharmacology and Treatment

Halogenation and topical corticosteroids: a comparison between the 17-butyrate esters of hydrocortisone and clobetasone in ointment bases

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SUMMARY

Clobetasone butyrate 0·05% (Eumovate), a halogenated topical steroid, was compared with hydrocortisone butyrate 0·1% (Locoid) which does not contain any halogen atoms. In the treatment of eczema there was no difference between the preparations, but in that of psoriasis the halogen-containing steroid was significantly more effective. Under normal circumstances neither preparation had any detectable effect on adrenal function, but with large doses under total-body polythene occlusion, circulating cortisol levels were reduced less by the halogenated than by the non-halogenated preparation.

Corticosteroids which contain a halogen atom are often considered to cause more adverse effects than the non-halogenated preparations with similar clinical efficacy. This study shows that this cannot be assumed for their ability to suppress cortisol levels.

During the development of corticosteroids, increased potency has often been achieved by incorporation of substituents (e.g. fluorine at the 6α or 9α position) into the natural hormone cortisol. Increases in topical potency have been associated with increases in topical lipophilic groups in the presence or absence of halogen substituents. However, there is no evidence to support the idea that fluorination *per se* is directly related to either the steroids' intrinsic therapeutic activity or their ability to produce local or systemic side effects.

Hydrocortisone butyrate 0·1% does not contain a halogen atom and published work suggested that it may have similar clinical activity to clobetasone butyrate 0·05% which does contain halogens (Munro & Wilson, 1975; Morley, Fry & Walker, 1976; Stevanovic, Wilson & Sparkes, 1977; Polano & Kaanar, 1973; Takino & Kobayashi, 1975; Yasuda, 1973). It was therefore of interest to compare the two preparations for efficacy and for the ability to affect the HPA axis following percutaneous absorption.

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PATIENTS AND METHODS

Comparison of clinical efficacy

Patients were consecutive out-patients with clinically similar bilateral lesions of eczema or psoriasis suitable for treatment with topical corticosteroids. The preparations were allocated at random to left or right side for application twice a day.

The study was double-blind. Methods of assessment and analysis were exactly as described previously (Sparkes & Wilson, 1974), with the effects of treatment being recorded at, or as near as possible to, 7 days from the start of the trial. The lesions were rated as healed, improved, static or worse, and whether one side had responded better than the other. The results were analysed by a sequential design (Armitage, 1960).

Comparison of effects on HPA axis in patients

Patients with psoriasis or eczema that was sufficiently widespread to need treatment in hospital were randomly allocated on admission to treatment with either hydrocortisone butyrate or clobetasone butyrate ointment. The trial was double-blind with ten patients in each group. HPA function was assessed by response to tetracosactrin at the beginning and end of the trial and also by frequent fasting early morning plasma cortisol estimations. The criteria for normality of the HPA axis were those laid down by Wood *et al.* (1965). The methods and assessment were as previously described by Allenby *et al.* (1975), in which the extent of the disease was graded from one to four.

Comparison of effects on HPA axis in volunteers

Hydrocortisone butyrate or clobetasone butyrate ointments were used by all volunteers with random allocation to first or second test week. The trial was double-blind.

In each test week five male volunteers with normal skin had blood samples for cortisol estimation taken at 9 a.m. each day from Monday to Friday. Over the 12-hour periods of Tuesday and Wednesday nights they applied 60 g of ointment to their trunk, arms and legs under polythene occlusion. After a gap of at least four weeks they used the comparison ointment in the same way.

RESULTS

Comparison of clinical efficacy

Forty-seven patients were included in the trial. The results are shown in Table 1 and Fig. 1. Thirty-three patients were admitted to the eczema section of the trial until the sequential analysis showed that a statistical difference at the 5% level between the two treatments would not occur. In the psoriasis section however, after fourteen patients had been treated, there was a significant difference in favour of clobetasone butyrate ($P<0{\cdot}05$).

TABLE 1. The number of times the clinician chose one preparation in preference to the other

	Clobetasone butyrate	Hydrocortisone butyrate	Equal	Total
Eczema	8	6	19	33
Psoriasis	7	0	7	14

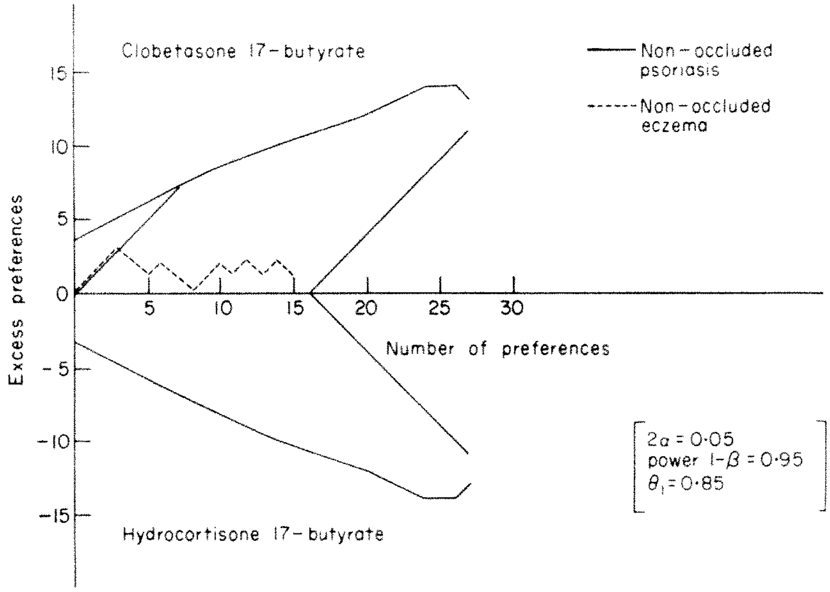

FIGURE 1. Sequential analysis of efficacy for clobetasone and hydrocortisone 17-butyrate

Comparison of effects on HPA axis in patients

The results are shown in Table 2. There were about the same number of patients with eczema as with psoriasis, but there was an uneven distribution between treatments. During the trial all dermatoses improved but only one was cleared. We presume, therefore, impaired barrier function in all but the one patient throughout the trial. All patients used around 200 g of ointment in the 7 days average treatment time. Eighteen of the patients had previously been treated with local steroids before admission and eleven of these had abnormal tetracosactrin tests on admission. In sixteen of these

TABLE 2.

	Number of patients								
	Psoriasis	Eczema	Total	HPA Function					
				Start					
				Abnormal			Normal		
Locoid	8	1	9	4			5		
				End					
					Abnormal	Normal		Abnormal	Normal
					2	2		1	4**
				Start					
				Abnormal			Normal		
Eumovate	3	7	10	7			3		
				End					
					Abnormal	Normal		Abnormal	Normal
					2	4*		1	2

*One not done at end.
**One had one day low cortisol.

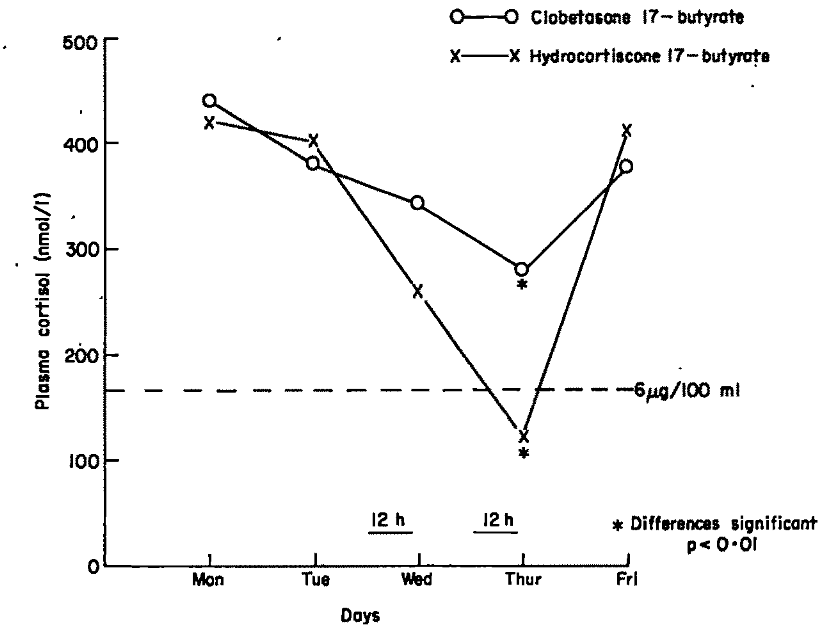

FIGURE 2. Plasma cortisol levels in volunteers receiving either clobetasone or hydrocortisone 17-butyrate.

recovery occurred by the time of discharge whereas in two of those normal on entry there was some abnormality at the end. Of those patients who had normal tetracosactrin tests at the start and end of the trial only one had an abnormally low 9 a.m. plasma cortisol level during treatment. We detect from these results no difference between Eumovate and Locoid.

Comparison of effects on HPA axis of volunteers

The results are shown in Fig. 2. All volunteers had normal 9 a.m. plasma cortisol levels on day one and two of each experimental week (control days). While using hydrocortisone butyrate one volunteer remained normal throughout, one was suppressed after the first night's occlusion and the other three suppressed after the second night's occlusion. When the same volunteers used clobetasone butyrate no significant depression of cortisol levels appeared. All levels returned to normal on the fifth day following a night without application of ointment.

DISCUSSION

When comparing the therapeutic activity of local preparations, statistical differences are not found if the dermatoses chosen for treatment are either easily healed or very resistant to treatment by both preparations. In this trial eczemas were cleared by both local steroids and no differences between them were shown; psoriasis was more slowly and less completely healed and showed the fluorinated steroid as statistically more effective. Application of the fluorinated or the non-fluorinated preparation to in-patients had similar effects on the HPA axis. Of the in-patients treated a number had abnormal HPA function on admission, presumably induced by out-patient local corticosteroid applications. When they were treated in hospital and the skin healed and barrier function was restored, the adrenal function of most of them either improved or returned to normal. Two patients, however, developed abnormal adrenal function during treatment though without total suppression. One of these was being treated with hydrocortisone butyrate, the other with clobetasone butyrate.

Under occlusive experimental conditions in normal subjects adrenal function suppression occurred with the non-fluorinated hydrocortisone butyrate but not with the clobetasone butyrate which is fluorinated. It has been shown that the number of halogen atoms is unrelated to a product's ability to produce side effects: clobetasol propionate contains the same number of halogen atoms as clobetasone butyrate but the former suppresses adrenal function (Allenby *et al.*, 1975; Staughton & August, 1975) and thins skin to a much greater extent than the latter (Munro & Wilson, 1975; Morley *et al.*, 1976; Sparkes, 1976; Winter & Wilson, 1976; Stevanovic *et al.*, 1977; Dykes & Marks, 1977); the degree of atrophy caused by steroids is not necessarily directly related to their anti-inflammatory potency (Smith, Wehr & Chalker, 1976); fluorination is not necessary for potent anti-inflammatory effect (Stewart *et al.*, 1973; Young, Yoxall & Wagner, 1977); and we now show that fluorination is not related to the ability to depress adrenal function.

We feel therefore that the word 'fluorination' associated with steroids should be discontinued as it tends, incorrectly, to be associated with efficacy and the ability to produce side effects.

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Factitious urticaria (dermographism): treatment by cimetidine and chlorpheniramine in a randomized double-blind study

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SUMMARY

The H1-antihistamine chlorpheniramine and the H2-antihistamine cimetidine, given alone and in combination, have been compared with placebo in twenty patients with factitious urticaria (dermographism), in a double-blind, randomized cross-over trial. Of these regimes, the combination was the only treatment which significantly reduced weal size, flare size and duration of weal, compared with placebo, although other treatments approached statistical significance. Continuation of the most effective of the four treatments in nineteen of the patients for a further 3 months without breaking the randomization code provided further evidence of the greater effectiveness of combined cimetidine and chlorpheniramine. No significant side effects were noted.

The dermographic response in patients with factitious urticaria is associated with release of histamine into skin (Greaves & Sondergaard, 1970) and has been widely supposed to mediate itching and the weal and flare reaction in this disorder. Recent studies using synthetic analogues of histamine, and specific histamine-H1 and histamine-H2 antagonists, have provided clear evidence for the existence of both H1 and H2 histamine receptors in human skin blood vessels (Marks & Greaves, 1977; Robertson & Greaves, 1978). Recently Commens & Greaves (1978) were unable to demonstrate significant benefit in combining H1 and H2 antihistamines in the treatment of chronic idiopathic urticaria. However, in ten patients Matthews *et al.* (1979) demonstrated a greater reduction in the weal response to two strengths of mechanical dermographic stimulus following combined treatment with cimetidine and chlorpheniramine over a 2-week period, than following administration of either drug alone. The present study in which we investigate the therapeutic effect of cimetidine, chlorpheniramine, a combination of these agents and placebo in factitious urticaria confirms and extends the Bristol workers' observations.

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PATIENTS AND METHODS

Patients

Twenty-one patients (seven males) have been studied, their ages ranging between 18 and 56 years (mean 31·2 years). The duration of the dermographism ranged from 2 months to 19 years (mean 3 years and 7 months). In addition to dermographism, five had chronic urticaria, one had chronic urticaria with angio-oedema, and another had chronic urticaria and papular urticaria due to insect bites. Informed consent was obtained from all patients.

Materials

The following oral medications were used: cimetidine—green film-coated tablets containing 200 mg cimetidine; chlorpheniramine maleate—4 mg tablets (supplied by Allen & Hanburys Ltd, Ware); placebo tablets to match cimetidine, and placebo tablets to match chlorpheniramine.

Methods

Existing medications, including antihistamines, were withdrawn for 1 week prior to the study. Of the twenty-one patients entered into the trial, fourteen patients completed the study for a full 4 months. Six withdrew after 5–6 weeks, and one withdrew 1 week after beginning the trial. The reasons for withdrawal were personal in four cases. Two found the hospital attendances too time-consuming, and one was so much better that he saw no reason for further attendance. Results are available on nineteen patients.

Patients were allocated on a random, double-blind basis to one of the following four treatment regimens:

(1) Cimetidine and chlorpheniramine (H1 + H2).
(2) Cimetidine and chlorpheniramine placebo (H2).
(3) Cimetidine placebo and chlorpheniramine (H1).
(4) Cimetidine placebo and chlorpheniramine placebo (placebo).

The dosage of cimetidine was 400 mg three times daily after meals and 400 mg at bedtime, and the dosage of chlorpheniramine was 4 mg three times daily after meals and 4 mg at bedtime. Each treatment regimen was taken for a 48 h period. The patients received the alternative treatments in random order, following a washout period of 5 days between each.

At the end of each 48 h period, patients returned to the out-patient clinic where their dermal weal response to a standard linear stroke was elicited on the upper back using a spring-loaded dermographometer. By applying graded pressures, using an arbitrary scale, the pressure required to produce a palpable weal of more than 2 mm in width was determined. The mean width of the weal and flare in response to this pressure was measured at 5, 10, 30 and 45 min after stroking. The time interval between stroking and onset of the weal and the weal disappearance time were also recorded.

After completing the four treatments, one of us (S.K.) judged which, if any, of the four treatments had produced the greater reduction in the weal response in each individual patient. This treatment was then continued for a further period of 3 months, without breaking the randomization code. During this period, patients were seen at 2 weeks, 1 month, 2 months and 3 months. At each of those attendances measurements of the dermographic weal were made as before, and the patients' subjective opinions on response to treatment sought and recorded.

RESULTS

Short-term study

Of the twenty-one patients entered into the study, one failed to return after the initial visit and was therefore excluded from analysis. Results for three patients were not analysed because of inadequate data. Weal size, flare size and duration of weal or flare have been rated on a 4-point scale (weal size or flare size: 0 = absent; 1 = mild (2 mm); 2 = moderate (2–4 mm); 3 = severe (4 mm). Duration of weal or flare: 0 = zero; 1 = short (30 min); 2 = moderate (30–60 min); 3 = severe (60 min)). Friedman's two-way analysis of variance by ranks was carried out to test for significant difference between treatments for weal size, flare size and duration. A significant difference between treatments was demonstrated for each of these measurements ($P<0·025$). The results are summarized in Table 1.

TABLE 1.

	P	H1	H2	H1+H2
Duration of weal	2·29	1·88	1·88	1·24
Flare size	2·47	1·71	2·53	1·24
Weal size	2·47	2·00	2·18	1·53

P = Placebo combination; H1 = chlorpheniramine; H2 = cimetidine; H1 + H2 = chlorpheniramine + cimetidine. Gradings as described in the text.

Duration of weal

The only treatment comparison which was significant at a 1% level using the Wilcoxon matched pairs signed ranks test was placebo *vs* combined cimetidine and chlorpheniramine ($P<0·01$). However, the following comparisons were significant at or below the 5% level (higher score given first): chlorpheniramine *vs* chlorpheniramine + cimetidine ($0·02>P>0·05$); placebo *vs* chlorpheniramine ($P=0·05$); cimetidine *vs* cimetidine + chlorpheniramine ($0·02>P>0·05$); placebo *vs* cimetidine ($P=0·05$).

Flare size

Four comparisons were significant at the 1% level: cimetidine *vs* chlorpheniramine ($P=0·01$); placebo *vs* chlorpheniramine ($P = 0.01$); cimetidine *vs* chlorpheniramine + cimetidine ($P<0.01$); placebo *vs* chlorpheniramine + cimetidine ($P<0·01$).

Weal size

The only treatment comparison which was significant at the 1% level was placebo *vs* chlorpheniramine + cimetidine ($P<0·01$). Two other comparisons were significant at or below the 5% level: placebo *vs* chlorpheniramine ($P=0·02$); cimetidine *vs* chlorpheniramine + cimetidine ($P=0·05$).

To summarize, only the combination of the H1 and H2 antagonists chlorpheniramine and cimetidine was effective in significantly reducing weal and flare sizes and duration of weal, compared with placebo. However, chlorpheniramine alone was more effective than cimetidine or placebo.

Choice of treatment for longer term therapy

Of the twenty patients who completed the four-treatment crossover study, a regimen could be identified which was clearly better than the others in nineteen. The distribution of treatments selected was:

combination of cimetidine and chlorpheniramine in thirteen; chlorpheniramine alone in five; cimetidine alone in one; placebo alone in none.

Longer term therapy

Nineteen patients commenced longer term treatment with the drug regimen chosen. One patient did not return for further assessment, but eighteen were reassessed at least once 4 weeks after starting the treatment, and thirteen remained on continuous full-dose treatment for the full 3 months. Cimetidine 400 mg four times daily combined with chlorpheniramine 4 mg four times daily was commenced in twelve patients. One patient withdrew from the trial when spontaneous weals appeared after 1 month, and a second defaulted after 2 months treatment. Changes in assessments of sizes of weals and flare and duration of weal during the course of treatment are listed in Table 2. For the ten patients who completed 3 months continuous therapy it is possible, in eight, to compare the scores recorded for each criterion after 3 months with that recorded at 1 month. In respect of weal size, three patients improved, four patients remained the same, and one deteriorated. The duration of the weal reduced in two patients, remained the same in five and became longer in one. The size of the flare improved in two, remained the same in one and deteriorated in one.

TABLE 2. Changes in weal and flare size and duration of weal in patients receiving treatment for up to 3 months

	Cimetidine + chlorpheniramine								
	Month 1			Month 2			Month 3		
Patient no.	W	F	D	W	F	D	W	F	D
1	1	2	1	1	2	1	0	2	1
2	2	2	2	—	—	—	1	1	1
5	0	0	0	—	—	—	0	0	0
6	2	2	2	0	0	0	0	0	0
8	—	—	—	1	1	0	0	0	0
10	0	0	0	1	1	1	1	1	0
12	2	2	2	2	2	2	2	3	3
13	2	2	2	0	0	0			
14	0	0	0	0	0	0	0	0	0
18	—	—	—	0	1	0	0	0	0
19	0	0	0	—	—	—	—	—	—
21	1	1	1	—	—	—	1	2	1
	Chlorpheniramine								
3	3	2	2	—	—	—	1	2	2
16	—	—	—	2	2	2	0	2	1
17	1	1	0	—	—	—	—	—	—
20	1	1	0	—	—	—	—	—	—
	Cimetidine								
15	0	0	0	0	0	0	0	0	0

W = Weal size; F = flare size; D = duration of weal. Gradings as described in the text.

In the smaller group of four patients who were given chlorpheniramine 4 mg four times daily alone, one defaulted, and one withdrew because of ineffectiveness of the treatment. Of the two who completed 3 months, a comparison of 3 months *vs* 1 month is only possible in patient 3. Weal size was reduced, but weal duration and flare size remained unchanged. The one patient who took cimetidine 400 mg four times daily for 3 months did not produce a dermographic weal in response to stroking during the longer term trial. However, the patient reported that some spontaneous weals had occurred between the out-patient visits.

Side effects

No significant side effects were noted, and routine laboratory tests were negative or normal.

DISCUSSION

The study was double-blind, but it became obvious at the end of the initial 4-week crossover trial that maximum suppression of wealing and subjective relief of symptoms were obtained with treatment no. 3 which, on subsequent breaking of the code, proved to be the combination of cimetidine and chlorpheniramine. No relief was obtained with treatment No. 4 (combined chlorpheniramine placebo and cimetidine placebo). According to Marks & Greaves (1977) there are two classes of histamine receptors in skin blood vessels, H1 and H2, which both play a part in mediating urticarial lesions due to histamine, and these workers suggested that combination therapy with H1 and H2 antagonists might prove advantageous in different varieties of urticaria. Results of a randomized double-blind trial of cimetidine and chlorpheniramine in chronic urticaria proved disappointing (Commens & Greaves, 1978), but the results of the present study confirm and extend the findings of Matthews *et al.* (1979) that combined treatment with these two drugs is more effective than either alone, or placebo, in symptomatic dermographism. That suppression by the combination is nevertheless incomplete is not surprising. These antihistamines administered systemically may not achieve sufficiently high concentrations in the vicinity of skin blood vessels to compete successfully with histamine at the receptor sites. Possibly other non-histamine mediators besides histamine may be involved in production of dermographism. Thus no single treatment is likely to prove ideal. Results of our longer term, double-blind study support the findings of the initial 4-week trial, and show that, at least over a 4-month period, there is no evidence of development of tolerance to the combined treatment. It must, however, be said that the long-term effects of cimetidine with or without H1 antihistamines remain to be established. Although no side effects appeared in our study, cimetidine should be administered for periods exceeding 3 months only with great caution. Studies of cimetidine in other skin disorders in which histamine is involved as a mediator are clearly needed.

ACKNOWLEDGMENTS

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Topical treatment of recurrent herpes simplex and post-herpetic erythema multiforme with low concentrations of zinc sulphate solution

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SUMMARY

The preventive effect of low concentrations of zinc sulphate solution in recurrent herpes simplex of the skin and oral mucous membrane is reported. Treatment with zinc sulphate solution of the skin at the site of the herpetic infection also prevents relapse of post-herpetic erythema multiforme.

For the skin, 0·025–0·05%, and for the oral mucous membrane, 0·01–0·025% zinc sulphate solution was used.

The aetiology of erythema multiforme is unknown. There are, however, different agents that may provoke the rash. The commonest known association is with a preceding herpes simplex infection (Forman & Whitwell, 1934; Champion, 1972). Generalized herpes simplex may be followed by erythema multiforme (Nasemann, 1974). Herpes simplex virus has been isolated from blisters in patients with erythema multiforme (Schmidt, 1962). The erythema multiforme rash appears 5–14 days after a herpes simplex episode (Forman & Whitwell, 1934; Nasemann, 1974).

Recurrent herpes simplex is a common and vexing problem confronting the practising physician, and a large number of treatments have been advocated (for review see Jarratt, Smith & Knox, 1979; Nahmias & Norrild, 1979; Spoor, 1979). Sauer (1948) recommended the use of a 20% zinc sulphate solution for the treatment of herpetic keratitis. DeRoetth (1963) treated herpetic keratitis successfully with 0·5% zinc sulphate solution.

For topical treatment, 0·2–1% zinc sulphate solution is recommended (Merck Index, 1968). For the skin, as well as for the oral mucous membrane, these concentrations are too strong and cause irritation, an unpleasant dryness and an emetic reflex. These side-effects can, however, be avoided with lower concentrations.

The present study was made in order to find out whether zinc sulphate solution—in such low concentrations that no side-effects appear—has any clinical effect on recurrent herpes simplex of the skin and oral mucous membrane. As erythema multiforme may be precipitated by a preceding herpes simplex infection it was, furthermore, of interest to investigate whether treatment with zinc

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sulphate solution only at the site of the herpetic infection would be sufficient to prevent relapse of the post-herpetic erythema multiforme.

PATIENTS AND METHODS

This study comprises five patients, aged 14–60 years, with recurrent herpes simplex (HS) and post-herpetic erythema multiforme (PHEM) lasting for 4–11 years, and twenty-five patients, aged 24–80 years, with recurrent HS lasting for 6–15 years.

In the patients with recurrent HS and PHEM the HS episodes occurred once a month or every second month and lasted for 4–14 days. The lesions were located on the skin close to the lips or on the lips. The PHEM rashes did not occur with every attack of HS. The PHEM rashes lasted for 4–6 weeks two to three times a year. They appeared 7–14 days after an HS episode and were, in three patients, located on the skin, nares and oral mucous membrane and, in two patients, only on the skin. The HS episodes with following PHEM rashes were associated with upper respiratory infections of long duration (4–6 weeks), whereas the HS episodes not preceding a PHEM rash were associated with upper respiratory infections of short duration (1–3 weeks).

In the twenty-five patients with recurrent HS, the lesions occurred once or twice a month or every second month and lasted for 6–10 days. They appeared on the skin around the lips (ten patients), on the nose and cheeks (six patients), on the shoulders (three patients), on the palm (one patient), and on the lips and the oral mucous membrane (five patients). The HS episodes were associated with upper respiratory infections of short duration (1–3 weeks).

In the present material the HS episodes did not occur except in connection with upper respiratory infections.

For the treatment of HS on the skin, a gauze compress soaked in a lukewarm zinc sulphate solution ($ZnSO_4.7H_2O$) was placed on the skin for about 10 min; for acute HS infection 0·025–0·05% and for the clinically normal skin at the site of the earlier HS lesion 0·05% zinc sulphate solution was used. The HS on the oral mucous membrane was treated with mouth rinses of zinc sulphate solution for 1–3 min in concentrations of 0·01–0·025% for acute HS infection and of 0·025% with a clinically normal mucous membrane.

In the patients with recurrent HS on the skin and oral mucous membrane, treatment was started during an acute infection once a day until the lesions disappeared. Maintenance treatment was given first once a week for a month and thereafter twice a month.

In all the patients with recurrent HS and PHEM the initial treatment was given during a PHEM rash, i.e. at least 7–14 days after the preceding HS episode. The clinically normal skin at the site of the earlier HS lesion was treated once a week as long as the PHEM rash lasted. Maintenance treatment was given twice a month. In the first two patients treated in this way, HS lesions relapsed in association with two successive upper respiratory infections of long duration. At the first relapse the HS lesions appeared only as tiny vesicles, whereas at the second relapse the lesions were of about the same extent as before the start of treatment. The site of the relapse was thus treated once a day until the lesions disappeared, once a week as long as the upper respiratory infection lasted and thereafter twice a month as maintenance.

This treatment was instituted in the other three patients with HS and PHEM at the first relapse of the HS infection even where this appeared only as tiny vesicles or as a colour change.

RESULTS

Treatment with the low concentrations of zinc sulphate solution prevented relapses in all patients with recurrent herpes simplex on the skin and oral mucous membrane during an observation period of 16–23 months. The upper respiratory infections occurred as frequently as before.

When the clinically normal skin at the site of the earlier herpetic lesions was treated in the patients with recurrent HS and PHEM, there were no relapses of the HS episodes associated with the upper respiratory infections of short duration or of the PHEM rashes. This treatment, however, did not prevent the HS episodes occurring in association with upper respiratory infections of long duration. In order to prevent these, they had to be treated in an acute stage. With this schedule of treatment it was possible to keep the five patients with recurrent herpes simplex and postherpetic erythema multiforme lesion-free during a period of 17–22 months. They suffered, however, from upper respiratory infections to the same extent as before treatment.

DISCUSSION

The recommended concentrations of zinc sulphate solution (0·2–1%) for topical use (Merck Index, 1968) cause considerable side-effects such as severe irritation and dryness of the skin and oral mucous membrane, and a strong emetic reflex. These side-effects are not provoked by the low concentrations of the zinc sulphate solution used for the skin (0·025%–0·05%) and for the oral mucous membrane (0·01–0·025%) in the present study. It has also been shown that these low concentrations are effective in preventing recurrences of herpes simplex infection in the skin and oral mucous membrane as well as of postherpetic erythema multiforme. The latter condition was controlled by treating only the skin at the site of the herpetic infection These observations confirm earlier studies on the topical effect of zinc sulphate solution in the treatment of herpes simplex infections (Sauer, 1948; DeRoetth, 1963; Tennican, 1979). In these earlier studies, however, 0·5% and higher concentrations of zinc sulphate solution were used.

In the investigations on the effect of zinc sulphate solution in herpetic keratitis (DeRoetth, 1963) and herpes genitalis (Tennican, 1979) a certain number of relapses have been reported. Such relapses were not seen in the present material of recurrent herpes simplex in the skin and oral mucous membrane. Only a study based on a large number of patients will tell to what extent initial treatment of an acute infection or of the clinically normal skin at the site of the herpetic infection and maintenance treatment are of importance for relapses of the HS infections. It is interesting to note that in the patients with recurrent HS and PHEM the initial treatment of the clinically normal skin at the site of the earlier herpetic infection prevented the relapses of the HS episodes associated with upper respiratory infections of short duration, but did not prevent the relapses of those associated with the upper respiratory infections of long duration. In order to prevent the latter, they had to be treated during the acute state.

Studies are under way to elucidate whether zinc sulphate solution may be used prophylactically to prevent recurrent herpes simplex provoked by agents other than upper respiratory infections. Although the material is small, preliminary results indicate that treatment of the clinically normal skin at the site of the earlier herpetic infection with 0·05% zinc sulphate solution before and during exposure to sun either prevents or decreases relapses of the herpes lesions provoked by sunlight. With an acute HS infection it is important to treat this daily until the lesions disappear and then continue with maintenance treatment.

Experimental investigations *in vitro* have shown that zinc ions irreversibly inhibit replication of herpes simplex virus type 1 in BSC-1 cells by selective inhibition of herpes simplex virus type 1 DNA polymerase (Gordon, Asher & Becker, 1975; Shlomai *et al.*, 1975; Fridlender, Chejanovsky & Becker, 1978). This effect of the zinc ions might explain the clinical effect of zinc sulphate solution in herpes simplex of the skin and mucous membranes.

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Case Reports

Report of an infant with GM_1 gangliosidosis type 1 and extensive and unusual mongolian spots

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SUMMARY

An infant with GM_1 gangliosidosis was found to have an eruption at birth consisting of extensive and unusual slate blue macules resembling mongolian spots. All areas of skin were involved except face, scalp, palms, and soles. A biopsy of a macule obtained at 5 months of age demonstrated melanocytic cells in the dermis consistent with mongolian spot but also a perivascular histiocytic infiltrate. At 8 months of age, absence of β-galactosidase activity was documented in both peripheral leukocytes and skin fibroblasts confirming the diagnosis of GM_1 gangliosidosis. The dermal histiocytic cells noted on skin biopsy were interpreted as a manifestation of this storage disease. The coexistence of the hyperpigmented lesions and the heritable enzyme defect was believed to be coincidental.

Deficiency of the enzyme beta-galactosidase may produce recognizable signs during the first 6 months of life. This early-onset disease is known as GM_1 gangliosidosis Type 1. Features that are commonly observed are: facial and peripheral oedema, full forehead, depressed nasal bridge, increased distance between the nose and upper lip, inactivity, and poor feeding. Hepatosplenomegaly and macular cherry-red spots develop. Additional findings that are usually present include beaking or hyperplasia of the superior aspect of the first lumbar vertebral body, vacuolization of renal epithelial cells which may be found in the urine, and vacuolization of peripheral white cells. Demonstration of decreased beta-galactosidase activity in cultured skin fibroblasts, urine, white blood cells or amniotic fluid cells establishes the diagnosis.

In GM_1 gangliosidosis Type 2, the enzyme deficiency produces neurological symptoms in association with normal facial features, organ size, and fundi, and the onset of the disorder is after 6 months of age. The mode of inheritance for both types is autosomal recessive.

CASE REPORT

The patient was the only child of healthy non-consanguineous black American parents. The pregnancy, labour, and delivery were uncomplicated.

At birth hyperpigmented skin lesions resembling mongolian spots were noted over the entire body

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sparing only the face, scalp, palms, and soles. The lesions were macular, grey to blue-black, non-hairy and varied tremendously in shape and size from a few millimetres to several centimetres (Fig. 1). Particularly large spots were present in the lumbosacral area and over the buttocks and left shoulder. Some were sharply demarcated but others had a feathery edge, and none blanched on pressure. As the child grew, the number of lesions did not change appreciably but all of them enlarged proportionately.

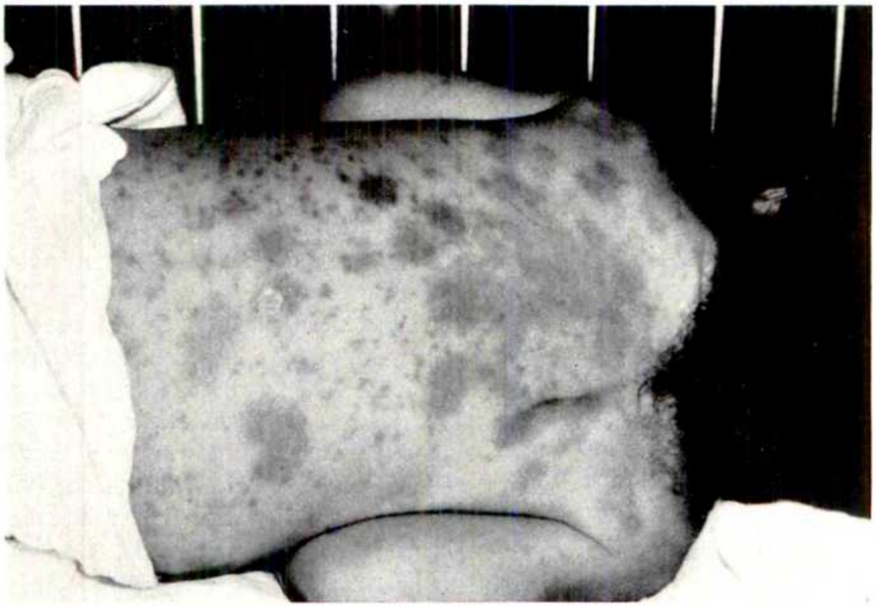

FIGURE 1. Back of patient showing marked variation in size and shape of macules.

Hepatomegaly was noted at age 5 months when he was hospitalized for pneumonia. His first admission to Children's Memorial Hospital was at age 8 months, also for pneumonia. In addition to the skin lesions, the prominent physical signs at this time were hypotonia, lethargy, full forehead, depressed nasal bridge, pale fundi with macular cherry-red spots, increased distance between the nose and upper lip, and hepatosplenomegaly (Fig. 2). Deformity of the first lumbar vertebral body was demonstrated on X-ray. The peripheral lymphocytes were vacuolated. Bone marrow examination showed many large lipid-laden histiocytes. Beta-galactosidase activity was absent in both peripheral leukocytes and skin fibroblasts. The child died at another hospital at 2 years of age. Autopsy permission was denied.

At 5 months of age a 3 mm punch biopsy was obtained from one of the hyperpigmented cutaneous macules. The epidermis was unremarkable but had an intensely pigmented basal cell layer. Numerous oval to spindle-shaped cells containing large amounts of melanin pigment were scattered throughout the upper dermis (Fig. 3). These cells morphologically resembled melanocytes. No naevus cells were present. A sparse perivascular inflammatory infiltrate consisting of histiocytes and lymphocytes was also noted. In several areas the predominant cell in the perivascular location was a histiocyte. Some of these histiocytes had a slightly foamy cytoplasm which was more abundant than normal (Fig. 4). Frozen sections were not available. An acid orcein stain demonstrated normal elastic fibres. Periodic acid-Schiff and alcian blue (pH 2·5) stains disclosed no abnormal material in cells or stroma.

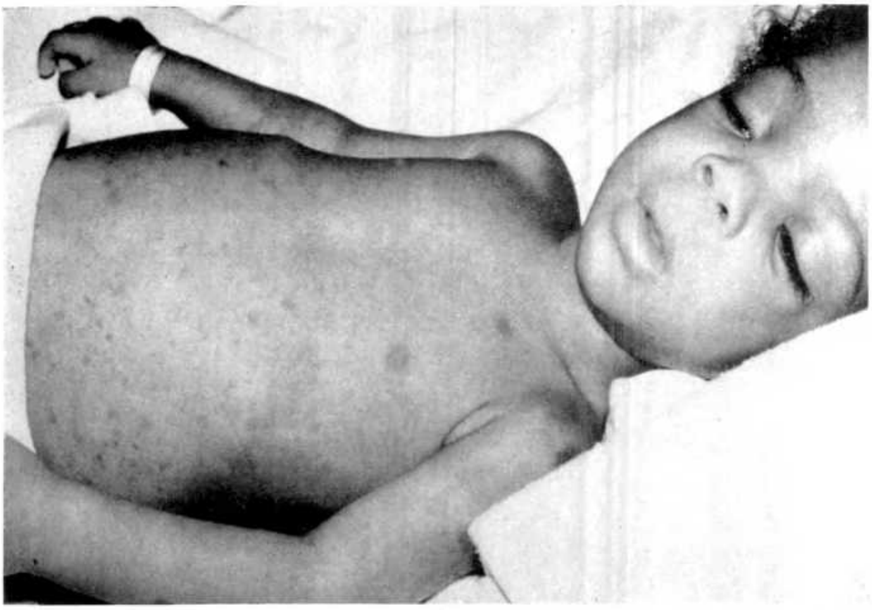

FIGURE 2. Patient at eight months of age with hepatosplenomegaly, depressed nasal bridge, full forehead and increased distance between the nose and the upper lip.

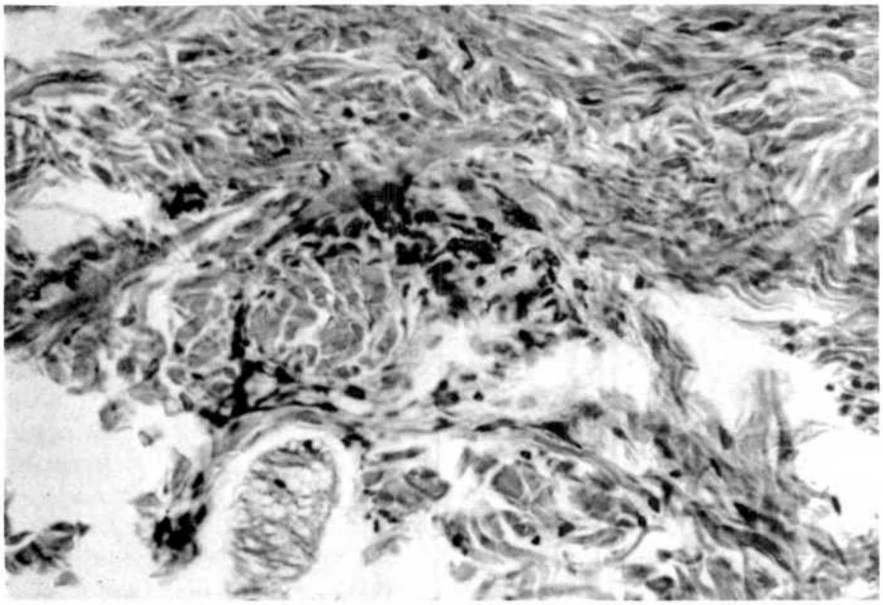

FIGURE 4. Mid-dermis showing hyperchromatic oval to spindle-shaped cells containing large amounts of melanin pigment (H & E × 175).

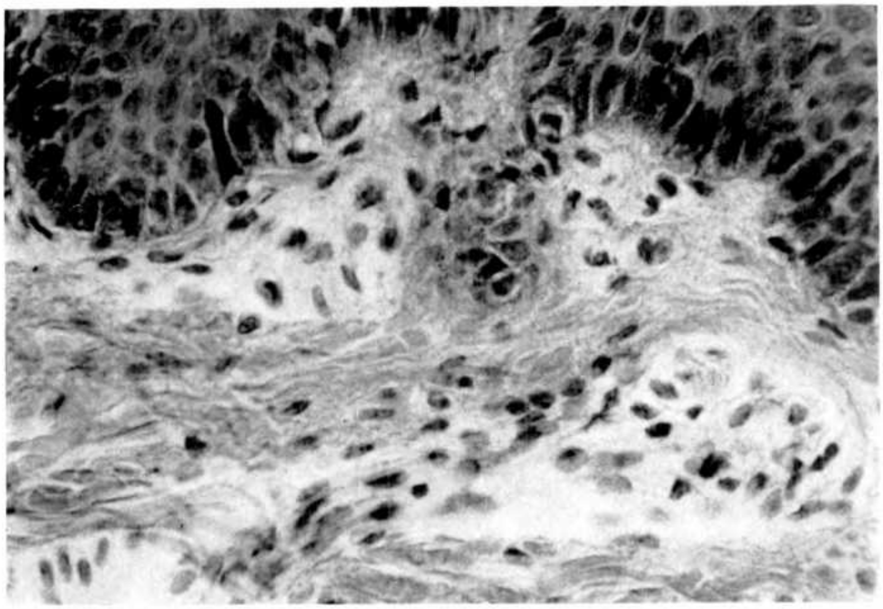

FIGURE 5. Subepidermal perivascular infiltrate composed of histiocytes with foamy cytoplasm (H & E × 300).

DISCUSSION

Dermatological manifestations appear to be uncommon in GM_1 gangliosidosis having been reported in only six infants, and recent review articles (Hirschhorn & Weissmann, 1976; O'Brien, 1976; Suzuki, Crocker & Suzuki, 1971) have not included a discussion of skin changes. An eczematoid facial rash associated with a macular rash on the body was present in two children (Landing, *et al.*, 1964; Suzuki, Suzuki & Chen, 1968). A detailed description was not presented, and no analysis of the skin was performed. Striking generalized cutaneous telangiectasia was observed in affected twins diagnosed at 7 months of age (Ginsburg & Long, 1977). They were said to have been present at birth, spared the mucous membranes, and could not be attributed to any other cause. Microscopic evaluation of the skin lesions was not performed. Telangiectasia appearing on the abdomen, pubis, thighs and face developed on another child at age two months (Hooft, *et al.*, 1969). The major dermatopathological abnormality in this child was marked swelling of the endothelial cells which contained vacuolated cytoplasm (Roels, *et al.*, 1970). Sections stained with toluidine blue and periodic acid-Schiff reagent were non-reactive.

The sixth of these children with GM_2 gangliosidosis Type 1 was noted to develop skin lesions at 8 months of age (Vissian, Manassero & Vaillaud, 1971). The lesions were described as rapidly enlarging, non-pruritic plaques distributed on the back, buttocks, posterior head and extremities. The plaques were reddish in colour, had ill-defined borders and were covered by dark crusts. Mucous membranes were unaffected. Histological examination of the affected skin demonstrated nests of moderately large histiocytes with clear or foamy cytoplasm in a dermal perivascular distribution. A few clear cells were also scattered throughout the dermal papillae. The hair follicles and sebaceous glands were normal; however, some of the eccrine secretory cells had an increased amount of cytoplasm. Histochemical studies on frozen sections included a negative Sudan black stain for lipids and a negative toluidine blue stain for metachromasia. Periodic acid-Schiff reaction in paraffin sections was positive in the dermal histiocytes; alcian blue stain was negative in the histiocytes but slightly positive in the eccrine gland epithelium suggesting accumulation of a mucopolysaccharide-like material in these cells. Multiple small cytoplasmic vacuoles, some of which were filled with a reticular substance, were found in the histiocytes by electron microscopy. These cells were said to resemble the histiocytes detected in other affected organs at *post mortem* examination.

The infant described in this report did not have skin lesions resembling those of previously reported affected infants. Nevertheless, a skin biopsy from one of the pigmented macules disclosed abnormal histiocytes indicative of an infiltrative process. Unfortunately, only a small amount of tissue was available and neither frozen sections nor electron microscopic studies could be performed. It seems likely, nonetheless, that these cells indicated the presence of a storage disease. Thus, this disorder can be added to the expanding list of enzyme defects reflected by abnormal histopathological findings in the skin.

The hyperpigmented macules found in our patient resembled mongolian spots clinically; however, the tremendous number of lesions and widespread involvement of skin were distinctly unusual. Although extensive ectopic mongolian spots are known to occur in some individuals, few such reports can be found in the literature apart from instances of naevus of Ota or naevus of Ito (Cole, Hubler & Lund, 1950; Pariser & Beerman, 1949). Hidano (1971), in a study of 9,996 healthy Japanese policemen, found persistent mongolian spots in 4·1%; in almost all the spots were solitary, but occasionally 2–3 spots were observed in a single individual. Most aberrant lesions were located on the back, shoulders and arms, and only rarely did they occur on the ventral surface of the body. Sparing of the flexor surfaces has also been noted by other authors (Brennerman, 1907; Pratt, 1953). In a series of 240 patients with naevus of Ota (Hidano *et al.*, 1967), six patients were found to have an additional persistent lesion on the back, buttocks or shoulder, and one patient had multiple spots in these areas.

Although there are several early studies documenting the incidence of mongolian spots in various ethnic groups (Adachi, 1903; El Bahrawy, 1922; Wateff, 1907), there is little information concerning infants with multiple lesions as regards their course and outcome (Ebert, 1928). More recent papers (Ayala-Uribe, 1970; Jacobs & Walton, 1976) have commented on the frequent occurrence of mongolian spots in the newborn infant but did not specifically mention infants with multiple lesions. A single patient, age 9 years, with progressive appearance of multiple blue spots has been reported (Carleton & Biggs, 1948); however, apart from a cranial abnormality, this child was well, and the nature of her pigmentary disorder was never conclusively settled.

The coexistence of a pigmentary abnormality and GM_1 gangliosidosis in this child is most likely fortuitous. However, the pigmentary lesions were extremely extensive and profuse for ordinary mongolian spots, and it was this clinical finding that prompted us to obtain a skin biopsy. Careful review of the haematoxylin and eosin sections disclosed a perivascular infiltrate of lymphocytes and histiocytes as well as scattered histiocytes in the dermal papillae. These cells are not usually present in biopsy specimens of mongolian spots (Lever & Schaumburg-Lever, 1975; Pinkus & Mehregan, 1969) and were, therefore, interpreted as a manifestation of the GM_1 gangliosidosis. However, the only biopsy obtained for histological evaluation was from hyperpigmented skin, and, therefore, we do not know if the normal-appearing skin contained a similar histiocytic infiltrate.

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Dapsone and severe hypoalbuminaemia in dermatitis herpetiformis

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SUMMARY

A case of severe hypoalbuminaemia in a patient on long-term dapsone treatment for dermatitis herpetiformis is described. The mechanism for this complication is uncertain, but increased intravascular albumin catabolism has been suggested.

In the United Kingdom the principal use of dapsone (4,4′-diaminodiphenyl sulphone) is in the treatment of dermatitis herpetiformis, when it is usually effective in doses up to 150 mg per day. In these doses adverse effects are uncommon, but red and white blood cell toxicity is a serious side-effect while other adverse reactions include neurological and psychiatric disorders, hepatitis, gastrointestinal upsets, and a form of the nephrotic syndrome (Graham, 1975). Recently the syndrome of severe but reversible hypoalbuminaemia has been reported in two patients on dapsone therapy for dermatitis herpetiformis (Kingham *et al.*, 1979) and we report here another example of this serious side-effect.

CASE REPORT

A 56-year-old Irish capstan setter presented with dermatitis herpetiformis in 1968 and was treated with dapsone 25 mg daily. In 1969 he suffered a perforated duodenal ulcer treated by oversewing and in 1971 he had a vagotomy and pyloroplasty. Following this operation he began to pass loose, pale, floating stools three to four times each morning.

In July 1975 he was referred to the London Hospital with a three week history of oedema of the lower limbs extending to mid-calf and bilateral periorbital oedema. There were also a right pleural effusion and a small amount of ascites, as well as a few dermatitis herpetiformis lesions on his elbows and buttocks and in his groins. By this time he was taking dapsone 25 mg twice daily. Haemoglobin concentration was 12·8 g/dl with normal indices, total white blood count was 7,800/mm^3 of which 13% (1014/mm^3) were eosinophils, and the erythrocyte sedimentation rate was 92 mm in the first hour. Serum concentration of albumin was 22 g/l (normal 37–52 g/l), and of globulin was 26 g/l (normal 24–30 g/l). There was no proteinuria and other biochemical tests were normal. A radiograph of the

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chest confirmed the right pleural effusion while barium meal and follow through showed no abnormality. Histological examination of a jejunal biopsy showed sub-total villous atrophy with stunted surface cells, a plasma cell and eosinophil infiltrate of the lamina propria, and increased thickness of the glandular layer. Fibreoptic gastroscopy showed prominent mucosal folds due to moderately severe gastritis confirmed histologically but without the features of Menetrier's disease. Daily fat excretion in the stools was normal (1·6 g per day) but gastrointestinal protein loss, measured with ^{51}Cr-chromic chloride, was 9% in the first two days and 4% in the second two days (normal: maximum loss of 1·5% in the first four days). This excess protein loss was attributed to the small bowel mucosal disease which was treated with a gluten free diet. Dapsone was continued at the same dose and the fluid retention was treated with frusemide.

Although the oedema partially cleared, by September 1975 ankle oedema and ascites were worse and he was readmitted to hospital. Haemoglobin concentration was by now 10·6 g/dl, total white count was 8,900/mm^3 with eosinophils comprising 1% and the erythrocyte sedimentation rate was 124 mm in the first hour. Serum concentration of the albumin was 24 g/l and plasma concentration of sodium was 117 mmol/l (normal 135–145 mmol/l). The ascitic fluid contained 23 g/l of protein and also bizarre, large, multi-nucleated cells that suggested an intra-abdominal malignancy. Sigmoidoscopy and barium enema were normal and histological examination of another jejunal biopsy showed that some improvement had taken place.

In late November he underwent an exploratory laparotomy for suspected disseminated intra-abdominal malignancy. There was no evidence of this, but there was oedema of the posterior abdominal wall, a retroperitoneal mass inferior to the pancreas and dilated lymphatic vessels. Biopsies were taken from the posterior abdominal wall, the retroperitoneal mass and the liver, while a duodenal lymph node and lymphatic vessel were also excised for histological examination. There was no neoplasia or other important abnormality in these specimens. His recovery after surgery was complicated by a pulmonary embolus to the left lung, arising from a deep vein thrombosis in the left leg. The consequent pulmonary infarction was complicated by abscess formation and then by empyema. The latter was initially treated by aspiration and antibiotics but eventually required rib resection and insertion of a drain.

Throughout most of this long admission the ascites and oedema persisted and were controlled with difficulty by large doses of diuretics. Corticosteroids were also given, starting with hydrocortisone after the laparotomy and subsequently prednisolone 5 mg daily. He remained on dapsone, initially 25 mg twice daily and later 25 mg once daily, until 6 weeks before discharge when it was stopped as the rash was quiescent and he was well established on a gluten free diet. Serum albumin concentrations remained below normal throughout the admission although just before discharge, when he had been off dapsone for 6 weeks, albumin reached its highest concentration to date (32 g/l) (Fig. 1.). At this time the gastrointestinal protein loss was 1·4% in the first 2 days and 0·9% in the second 2 days.

In late August 1976 he was readmitted with a right femoral vein thrombosis that was treated with warfarin. Ascites was still present despite diuretics and the dermatitis herpetiformis rash had returned so that during April he had restarted dapsone 50 mg daily, two or three times per week. Serum albumin concentration was again very low at 20 g/l and plasma sodium concentration was 126 mmol/l.

In early October 1976 he was again admitted. On this occasion with an incarcerated left inguinal hernia causing intestinal obstruction. The incarceration was managed conservatively but the hernia was later repaired under epidural anaesthesia. Ascites and oedema of the legs were present and he was still taking dapsone 50 mg daily two or three days a week. Just before this admission prednisolone had been increased to 15 mg daily to try to correct the hypoalbuminaemia which by this time was 18 g/l.

He was treated with intravenous infusions of plasma protein fraction, a blood transfusion, and larger doses of diuretics. He remained on a gluten-free diet and, for 1 month, on prednisolone 15 mg

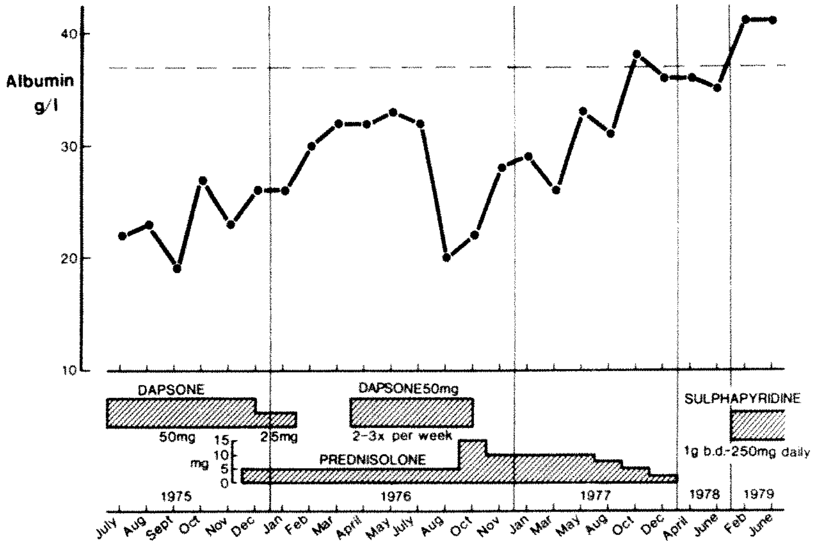

FIGURE 1. Changes in serum concentration of albumin in relation to treatment with dapsone, prednisolone and sulphapyridine. The broken line represents the lower limit of normal of serum albumin concentration.

daily, but dapsone was stopped not only because the rash was quiescent but also because it was suspected of causing the hypoalbuminaemia in some way. During this admission serum albumin concentration slowly rose and was 27 g/l on discharge, by which time the ascites and oedema were more or less under control.

He remained well for 8 months on prednisolone 10 mg daily while serum albumin concentrations rose slowly, although they never reached normal. He then developed severe mid-dorsal back pain and radiographs of the dorsal spine showed generalized osteoporosis and collapse of D7, D8 and D9 vertebral bodies. These changes were attributed to chronic steroid ingestion and were treated symptomatically and by slow reduction in the dose of prednisolone. At this time there was no peripheral oedema and only a trace of ascites, and serum concentration of albumin was 33 g/l. Since then serum albumin has continued to increase slowly and has been normal throughout the last 12 months. Ascites and oedema have not recurred since the last admission and diuretics are no longer required. Moreover, the patient has not taken dapsone for the last 3 years, the rash being controlled with sulphapyridine, initially in a dose of 2 g each day reducing to 250 mg daily recently.

DISCUSSION

This patient with dermatitis herpetiformis had taken dapsone 25–50 mg daily for 8 years without adverse effects when oedema and ascites suddenly developed and he was found to have severe hypoalbuminaemia. At first this was attributed to a protein-losing enteropathy resulting from partial atrophy of the small bowel mucosal villi, and in support of this a radiolabelled protein losing study demonstrated a marked increase in faecal protein loss (13% in four days). However, on a gluten-free diet the small bowel mucosal villi improved and gastrointestinal protein loss returned almost to normal, but serum albumin concentrations remained low. Instead, dapsone seems to have been the cause, with a steady improvement in general condition and a slow return of serum albumin concentration to normal, with loss of ascites and oedema, after dapsone was stopped 3 years ago.

The association between dapsone and hypoalbuminaemia in this patient was not suspected until he had become seriously ill and had undergone a laparotomy, developed a lung abscess and empyema, and several vertebral bodies had collapsed, probably in part related to chronic steroid usage. Two other patients in whom this complication of dapsone has been described were also extremely ill before the diagnosis was made (Kingham *et al.*, 1979) and a third patient, in whom this complication has been suspected only in retrospect, died with hypoalbuminaemia while still taking dapsone (Young & Marks, 1979). Our patient, like these other three, had been taking dapsone for many years without apparent adverse effect, a factor that contributed to the delay in diagnosis in all four patients.

Kingham *et al.* (1979) suggested that the hypoalbuminaemia was due to a marked increase in albumin catabolism when taking dapsone. They measured albumin turnover in one patient and suggested that the increased fractional catabolic rate of albumin was related to the high degree of binding of dapsone to albumin (Riley & Levy, 1973) where it might act as a hapten and cause immunological destruction of albumin. Indirect support for this immunological mechanism is perhaps the lack of relationship of this side-effect to the dose used, in that the patients described were severely ill on doses of dapsone that ranged from a maximum of 150 mg daily to a minimum of 50 mg two or three times a week. Albumin turnover studies were not performed in our patient and we are not able to advance the understanding of the mechanism of dapsone-induced hypoalbuminaemia. Instead, our patient illustrates the severity of this uncommon syndrome and we suggest, therefore, that, whenever possible, a gluten-free diet should be tried to control the rash, as well as the abnormal jejunal mucosa, in dermatitis herpetiformis (Fry *et al.*, 1973), as this will allow dapsone to be stopped in some patients (Cooper *et al.*, 1978).

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Brief Communications

Ketotifen and terbutaline in urticaria

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Both immunological and non-immunological factors can cause mast cells and basophils to release mediators capable of producing urticaria (Monroe & Jones, 1977). Cellular cyclic AMP may modulate both these processes (Orange & Austen, 1971; Parker, Sullivan & Wedner, 1974).

Terbutaline, a β_2 adrenoceptor agonist, probably inhibits mast cell degranulation by increasing cyclic AMP levels. Ketotifen, a benzocycloheptathiopene derivative, increases cAMP levels and also inhibits release of histamine from rat peritoneal mast cells by compound 48/80 *in vitro* (Martin & Römer, 1978). Both these drugs have been used in asthma.

Some patients with urticaria respond poorly to antihistamines, and in these patients substances other than histamine which are released from mast cells and basophils may be more important. It seems reasonable to treat such patients with a combination of ketotifen and terbutaline, both of which tend to increase cellular cAMP and inhibit mast cell degranulation.

METHODS AND RESULTS

Twelve patients (six female, six male, aged between 20 and 65 years) with chronic idiopathic urticaria of 6 months to 9 years duration, who had failed to respond to antihistamines, were treated with a combination of ketotifen and terbutaline. Ketotifen 1 mg and terbutaline 5 mg were both given three times a day. The drugs were taken separately with 1 h between the two drugs. Two patients with physical urticaria, one (male, 58 years) with marked dermographism and one (female, 19 years) with cold urticaria, where both antihistamines and hydroxyzine had failed, were similarly treated. Patients were seen in the clinic every 2–3 weeks and were advised to observe any improvement in pruritus, severity of urticaria, time of clearance and any side effects.

Of the twelve patients with chronic urticaria, four patients were started with ketotifen alone. In these patients, the pruritus improved but the urticaria did not clear. Two weeks later terbutaline was added and within 3–4 days the urticaria almost completely cleared. One of these four patients complained of fine tremor and occasional palpitation for about a week. The other eight patients with chronic urticaria were treated from the start with both drugs. In four patients the urticaria cleared within 3–4 days. In one patient of the remaining four the urticaria flared up and there was no response, even after 1 week of treatment. In three patients treatment had to be discontinued within the first 2 days due to tremor and/or palpitations. These side effects were most likely due to terbutaline.

In three patients, both drugs were discontinued after 8 weeks, but the urticaria returned within about 2 days. In one of these three patients the urticaria was much milder and remained so without treatment. Both patients with physical urticaria became symptom free within 1 week of treatment.

DISCUSSION

Thus, the combination of ketotifen and terbutaline was shown to be effective in ten out of fourteen patients with urticaria. Kennes, De Maubeuge & Delespesse (1977) found terbutaline to be helpful in

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urticaria, although the maximum therapeutic effect took about a month, and we have found it to be relatively ineffective on its own (unpublished data). Part of the beneficial effect of ketotifen could be due to its antihistamine property (Martin & Römer, 1978), but our patients had failed to respond to antihistamines. It seems more likely that the combined effect of ketotifen and terbutaline, acting at two different sites, was enough to raise the cyclic AMP levels in the mast cells, thus having a stabilizing effect.

The combination of ketotifen and terbutaline may thus be a suitable therapy for urticaria.

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Serum immunoglobulins in lichen planus

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Serum immunoglobulin levels were assayed in a group of thirty-one patients with lichen planus on first presentation and after resolution.

Stankler (1975) found a deficiency of IgA and IgM levels in thirteen consecutive adults with lichen planus. Jacyk & Greenwood (1978), in a series of forty-two Nigerian patients, confirmed the deficiency of IgM relative to controls, but found no difference in IgA and IgG levels. It has not been established whether these levels are transient or whether a more permanent immunological abnormality exists in patients with lichen planus. In order to investigate this further, all patients presenting to this hospital with lichen planus had their serum immunoglobulins assayed, starting in November 1978. The patients in this series represent the first thirty-one of the above who have been followed until disease resolution, after which the assay was repeated. Disease resolution was arbitrarily defined as the absence of oral and cutaneous lesions other than residual, impalpable hyperpigmentation.

The thirty-one patients comprised seventeen males and fourteen females with a mean age of 47·8 years (range 10–82 years). The time (mean ± s.d.) of the first assay was 4·5 ± 3·7 months and of the final assay 10·5 ± 4·9 months after the onset of symptoms. A further fourteen patients who had suffered from lichen planus at least 2 years previously were selected at random from the departmental records and their immunoglobulin levels were measured. None of these patients still had active disease. They comprised five males and nine females with a mean age of 54·9 years (range 35–72 years). The immunoglobulins were measured nephelometrically using a Technicon autoanalyser.

The results are shown in Table 1. Patients with 'previous' lichen planus had levels within the normal range. Patients with 'recent' lichen planus showed little variation from the normal apart from slightly low IgM levels when the disease was active. However, comparing levels for individual patients during disease activity and after resolution there was a clear rise in IgG and IgM levels (paired t-test; $P<0{\cdot}001$ and $P<0{\cdot}01$ respectively). There was a less significant rise ($P<0{\cdot}05$) in IgA levels.

This study seems to suggest that there is some, probably minor, disturbance of serum immunoglobulins which reverts to normal as the skin clears. Whether this disturbance precedes, parallels, or indeed follows the onset of lichen planus cannot be deduced at this stage.

TABLE 1. Serum immunoglobulin levels in g/l (± s.d.) in patients with recent and previous lichen planus

	Recent lichen planus		Previous lichen planus (n = 14)	Normal range	Significance: paired t-test
	Active	Resolved			
IgG	10·9 ± 2·4	12·0 ± 2·7	12·2 ± 2·9	5·0–16·0	$P<0{\cdot}001$
IgA	2·0 ± 0·6	2·2 ± 0·9	2·5 ± 1·1	1·0–3·5	$P<0{\cdot}05$
IgM	0·6 ± 0·3	0·9 ± 0·6	1·2 ± 0·6	0·5–1·7	$P<0{\cdot}01$

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Review Article

Cutaneous manifestations of paraproteinaemia. Part II

In the first part of this review (Russell Jones, 1980), the associations between paraproteinaemia and cutaneous disorders were classified on a pathogenetic basis into five groups: specific infiltrates (Group I), light chain deposition (Group II), quantitative effects of paraprotein production (group III), immunological effects of paraprotein production (group IV) and associations of indeterminate aetiology (group V). Vasculitis and related processes which depend upon the complement-fixing ability of paraproteins, were included in group IV, and a distinction was made between paraproteins which activate complement directly and paraproteins with antibody activity in which complement activation results from immune complex formation. Also, the importance of establishing a pathogenetic role for the paraprotein was emphasized, since vasculitis and paraproteinaemia may co-exist without being causally related.

Similar considerations apply to the conditions discussed in the second part of this review (Table 1). Other types of immunological activity and conditions where the underlying mechanism is unknown are reviewed. Since monoclonal gammopathies occur in approximately 1% of the population, many disorders may co-exist with paraproteinaemia merely by chance, so group V associations are limited to conditions which show a characteristic type of paraprotein (e.g. lichen myxoedematosus), or where the association occurs so often that the paraprotein becomes a marker for the condition (e.g. systemic capillary leak syndrome and necrobiotic xanthogranuloma).

Lipid metabolism

The association between paraproteinaemia, serum lipids, and cutaneous xanthomata is complex since paraproteins exhibit a number of properties which will interfere with lipoprotein metabolism. Usually sera from patients with myeloma and Waldenstrom's macroglobulaemia show lowered or normal serum lipids and this correlates with the paucity at autopsy of significant atheroma (Spain *et al.*, 1956). Riesen, Noseda & Butler (1971) have demonstrated that hypolipidaemia is associated with lipoprotein binding activity, and postulate the formation of lipoprotein-paraprotein complexes which are cleared more rapidly from the circulation than is lipoprotein alone. They point out that HDL and LDL have different antigenic specificities, that VLDL is immunochemically identical to LDL, and that the paraproteins in their series showed binding with LDL and VLDL only. No antibody activity directed against HDL was demonstrated. Treatment of patients with M-component disorders produces a rise in the beta-lipoprotein level and a corresponding normalization of serum lipids (Shulman *et al.*, 1976).

The term diffuse plane xanthomatosis (DPX) indicates the presence of widespread xanthomatous skin deposits in which the lesions are typically flat or slightly elevated and vary greatly in size, often producing large areas of orange or yellow discolouration. The eruption is characteristically associated with lymphoproliferative disorders (Lynch & Winkelmann, 1966) and has been reported in association with both benign monoclonal gammopathies (Russell Jones *et al.*, 1979) and multiple myelomatosis

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TABLE 1. Mechanisms (continued from Part I)

Immunological effects of paraprotein: lipid metabolism	
Anti-lipoprotein activity: formation of lipoprotein-paraprotein complexes	(1) Increased clearance rate: hypolipidaemia (2) Deposition: diffuse plane xanthomatosis—DPX (3) Reduced clearance rate: hyperlipidaemia; DPX ± other xanthomata
Antiheparin activity: reduced lipoprotein lipase activity	Hyperlipidaemia: tuberous xanthomata
Immunological effect of paraprotein production: miscellaneous binding activities	
Antiflavin activity: xanthoderma	
Antitransferrin activity: haemochromatosis	
Anticopper activity: corneal deposits	
Possible immunological effects of paraprotein production	
Phagocyte inhibition by IgA paraproteins	(1) Increased susceptibility to infections (2) Pyoderma gangrenosum (3) Subcorneal pustular dematosis
Associations of unknown aetiology	
(1) Lichen myxoedematosus	
(2) Systemic capillary leak syndrome	
(3) Necrobiotic xanthogranuloma	
(4) 'Japanese' syndrome	
(5) Other markers of internal malignancy Pruritus Erythema gyratum repens, etc.	

(Taylor *et al.*, 1978). Patients with DPX are of particular interest since xanthoma formation seems to occur independently of the serum lipid levels which are often normal. A characteristic finding in the serum of patients with paraprotein associated DPX is lipoprotein-paraprotein complexes. Although it is not certain how the formation of such complexes leads to xanthoma deposition in the dermis, it is likely that deposition occurs in a manner analogous to immune-complex vasculitis. The rate of lipid deposition would then be determined, amongst other things, by the properties of the complex rather than the serum lipid levels, and this would account for the observation that many cases are normolipaemic. Other cases of DPX, particularly those associated with IgA myeloma proteins, exhibit hyperlipidaemia as well as complexing. The mechanism of hyperlipidaemia is not clear. It may be that it results from reduced clearance of the lipoprotein-paraprotein complexes (Beaumont, Jacotot & Beaumont, 1965), but such complexes are usually cleared more rapidly (Riesen *et al.*, 1971). Further, Marien & Smeenk (1975) were able to demonstrate that the paraprotein in their case bound HDL only, whereas the patient exhibited a Type IIA hyperlipidaemia with high levels of LDL. Such observations indicate that the formation of circulating complexes is only one mechanism by which paraproteins with antilipoprotein activity interfere with lipid metabolism, and that hyperlipidaemia is not a prerequisite for the development of skin lesions in DPX. This explains why the lipid composition of xanthomata in DPX does not correspond with the serum lipids (Kodama, Nakagawa & Tanioku, 1972), and also accounts for the observation that these xanthomata may respond to therapy for the myeloma, but not for the hyperlipidaemia (Moschella, 1970).

A variety of techniques have been used to identify and analyse lipoprotein-paraprotein complexes.

In some cases abnormal bands are visualized on lipoprotein electrophoresis. Complexes can be extracted from serum by preparative ultracentrifugation and in a few instances simply by cryoprecipitation (Beaumont, 1969; Kodama *et al.*, 1972; Wilson *et al.*, 1975; Taylor *et al.*, 1978). Ultracentrifugation at high salt density will separate the complex into its individual components and the isolated paraprotein and lipoprotein fractions can then be used in recombination experiments to demonstrate the specificity of the binding. This appears to be a variable feature. Some paraprotein fractions bind beta-lipoprotein only (Aubert *et al.*, 1967; Beaumont, 1968; Taylor *et al.*, 1978), others bind alpha-lipoprotein only (Marien & Smeenk, 1975), whilst others appear to combine with both (Kodama *et al.*, 1972).

Occasional failure to demonstrate complexes in the serum of patients with paraprotein associated DPX may depend upon the quantity in the circulation and the sensitivity of the methods used. Circumstantial evidence for such complexes may be provided by complement abnormalities (Jordon *et al.*, 1974; Linscott & Kane, 1975; Russell Jones *et al.*, 1979), and a few patients with DPX exhibit petachiae within the xanthomatous area (Russell Jones *et al.*, 1979).

When hyperlipidaemia is associated with M-component diseases, other varieties of cutaneous xanthomata are seen such as tuberous xanthomata (Savin, 1965; Beaumont, 1968; Wilson *et al.*, 1975), eruptive xanthomata (James & Harland, 1968) and even palmar xanthomata (Roberts-Thompson *et al.*, 1975). It is important to distinguish these groups as the mechanisms involved are liable to be different. Thus paraproteins with antiheparin activity interfere with lipid metabolism as heparin is essential for the activity of certain enzymes, including lipoprotein lipase (Glueck, Mackenzie & Glueck, 1972). Such cases exhibit defective lipoprotein turnover, and hyperlipidaemia. Cutaneous deposits can occur. The case reported by Wilson *et al.* (1975) showed a high level of intermediate density lipoproteins, atherosclerosis and tuberous xanthomata. Evidence of such processes is not found, however, in all cases. Roberts-Thompson *et al.* (1975) reported a case of polymeric IgA myeloma and Type IV hyperlipidaemia with eruptive and palmar xanthomata in whom anti-lipoprotein and antiheparin activity was specifically excluded.

In conclusion, therefore, M-component disease can occur with low, normal or elevated serum lipids. Antilipoprotein activity with formation of lipoprotein-paraprotein complexes is associated particularly with diffuse plane xanthomatosis. Other varieties of cutaneous xanthomata are seen if hyperlipidaemia is present as well. Hyperlipidaemia can result from antiheparin activity. It may also result from reduced clearance of lipoprotein-paraprotein complexes, but the existence of other as yet unidentified mechanisms also seems likely.

Miscellaneous binding activities

As discussed previously, well recognized clinical features are associated with paraproteins which display antibody activity towards IgG, red cells, clotting factors or lipoprotein. Occasionally, monoclonal proteins bind smaller molecular haptens such as dinitrophenyl derivatives, phosphorylcholine, dextrans and nucleic acids (Seligmann & Brouet, 1973). Usually these interactions do not produce overt clinical manifestations, but occasionally unique syndromes can result. Farhangi & Osserman (1976) reported a patient with xanthoderma due to an IgG myeloma protein with anti-flavin activity. The purified IgG was bright yellow and the acid-dissociated chromophore was identified as riboflavin. Similarly, Westerhausen *et al.* (1972) reported a patient with haemochromotosis who was found to have a high serum iron and an IgG myeloma protein which bound transferrin to produce an abnormal brownish protein with a sedimentation coefficient of 9·9 S. Finally there have been two reports of paraproteins with a high affinity for copper. Goodman, Rodgerson & Kauffman (1967) reported a patient with IgG myeloma and hypercupraemai who showed brownish discolouration of the cornea, and Lewis, Falls & Troyer (1975) reported a patient who presented with blurring of vision due to deposition of copper within the cornea. Further studies demonstrated that most of the copper was circulating bound to the IgG myeloma protein (Lewis *et al.*, 1976) and therapy with chelating agents was ineffective.

Microbial infection

Increased susceptibility to infection is a feature of patients with plasma cell dyscrasias and is included as a group III association since it depends at least in part upon reciprocal depression of other immunoglobulin classes (Broder *et al.*, 1975), which in turn is a reflection of total B cell mass. Apart from immunosuppressive therapy, several other factors may influence host susceptibility including the class of paraprotein. Polymorphonuclear leucocytes carry IgA receptors (Lawrence, Weigle & Speigelberg, 1975) and aggregated IgA has been shown to inhibit phagocytosis of IgG complexes by polymorphs (Wilton, 1978), and to interfere with neutrophil chemotaxis *in vitro* (Van Epps & Williams, 1976). Other classes of paraprotein did not exert these effects. Impaired chemotaxis *in vitro* is associated with increased susceptibility to bacterial infection *in vivo*, and cutaneous manifestations include chronic folliculitis, recurrent abscesses and other pyodermatous skin lesions (Snyderman, Pike & Altman, 1975). Whether such mechanisms account for the different patterns of infection seen with different classes of paraprotein is not known (Hobbs, 1969; Allen, 1970), but would clearly repay further study.

Finally paraproteins which bind complement could interfere with host resistance since complement consumption by an M-component could lead to depletion of C_3b and other complement factors necessary for normal opsonization and phagocytosis. This consideration is probably of theoretical importance only since increased susceptibility to infections seems to be associated mainly with hereditary rather than acquired complement deficiency states (Agnello, 1978).

Pyoderma gangrenosum

Pyoderma gangrenosum (PG) is characterized clinically by areas of cutaneous ulceration which exhibit a blue undermined edge, show a variable response to oral steroids and heal to produce areas of cribriform scarring. Although there is no evidence implicating micro-organisms in the causation of this condition, early lesions of PG often resemble pyogenic infections morphologically and are characterized histologically by a heavy infiltrate of polymorphs. Associated disorders include inflammatory bowel disease, myeloproliferative disorders, paraproteinaemia and inflammatory arthritis which may be seropositive or seronegative (Holt, 1979; Hickman & Lazarus, 1980). A wide range of immunological abnormalities have been described in PG, including abnormal monocyte function (Norris *et al.*, 1978) and defective polymorph function in nine out of fifteen patients with PG (Holt *et al.*, 1980) and the beneficial effect of clofazamine in this condition may depend upon the ability of this drug to enhance neutrophil function (Michaelsson *et al.*, 1976; Thomsen & Rothenberg, 1979). It is therefore of interest that the majority of paraproteins associated with PG belong to the IgA class (Van der Sluis, 1966; Thompson *et al.*, 1973; Holt *et al.*, 1980), since this particular class of immunoglobulin is known to inhibit neutrophil function *in vitro* (Van Epps & Williams, 1976; Wilton, 1978). Of the three patients with PG and IgA myeloma reported by Holt *et al.* (1980) one showed reduced polymorph chemotaxis *in vitro*, another showed delayed polymorph migration using an *in vivo* skin window technique and the third had an abnormal bactericidal capacity and a reduced NBT test. Unfortunately the ability of these myeloma proteins to inhibit neutrophil function *in vitro* was not specifically tested, but it is known that therapy for the paraprotinaemia may produce resolution of the cutaneous lesions (Moller, Waldenstrom & Zettervall, 1978). These observations raise the intriguing possibility that immunological competence in patients with PG may be compromized by paraproteins which block cell surface receptor sites on neutrophils and/or monocytes.

Similar mechanisms may operate in cases of PG-associated arthritis. Soluble immune-complexes, particularly IgA containing complexes, are known to inhibit polymorph chemotaxis (Leung-Tack, Maillard & Voisin, 1979; Todd Davies *et al.*, 1977; Ito *et al.*, 1979), neutrophil chemotaxis is defective in patients with rheumatoid arthritis (Mowlat & Baum, 1971), and reduced chemetaxis *in vitro* or defective polymorph migration *in vivo* has been documented in cases of PG-associated arthritis (Holt *et al.*, 1980). It would, however, be difficult to account for all cases of pyoderma gangrenosum on this basis. Neutrophil chemotaxis may be abnormal in patients with PG-associated leukaemia (Shore, 1976), and this may reflect an intrinsic cellular defect rather than surface coating with complexes or paraproteins. Furthermore, serum from one patient with PG actually enhanced leukocyte mobility (Jacobs & Goetzl, 1975). It therefore seems likely that cutaneous lesions of PG represent the end-point of several different pathological processes. Investigation of such mechanisms does however offer a more rational approach to the classification of this poorly defined clinical entity.

Sub-corneal pustular dermatosis

The clinical and histopathological hallmarks of Sneddon-Wilkinson disease have been recently reviewed by the two eponymous authors who originally delineated this rare cutaneous disorder (Sneddon & Wilkinson, 1956; Sneddon & Wilkinson, 1979). The eruption is distinguished from pustular psoriasis by its flexural distribution, sub-corneal pustulosis which spares the epidermis below, and a favourable response to sulphapyridine or dapsone. The pathogenesis of the disorder is unknown though several authors have reported an association with paraproteinaemia. Although rare, it is important to recognize the association as myeloma may develop several years after the onset of the rash (Schnitzler *et al.*, 1977). Apart from one case associated with an IgG cryoglobulin (Sneddon & Wilkinson, 1979) the other gammopathies have belonged to the IgA class (Wolff, 1971; Dahlouk *et al.*, 1975; Schnitzler *et al.*, 1977; Cream, Grimes & Roberts, 1977), which again illustrates the association between pustular skin lesions and a class of immunoglobulin known to inhibit neutrophil function. Indeed the patient reported by Wolff (1971) demonstrates that sub-corneal pustular dermatosis, pyoderma gangrenosum, and IgA paraproteinaemia may co-exist. Clearly, however, the two conditions are distinguished by the sub-corneal location of the pustules in Sneddon-Wilkinson disease, and the dermal location of the neutrophilic infiltrate in pyoderma gangrenosum, so other factors such as the nature of the chemotactic stimulus must be important. Non-infective chemotactic stimuli such as tissue injury may act as a focus for polymorph activity in patients with pyoderma gangrenosum, whilst chemotactic substances in the stratum corneum may be relevant in Sneddon-Wilkinson disease, as in pustular psoriasis (Tagami & Ofuji, 1977). Detailed investigation of individual cases is however required to test these possibilities further, for it is also possible that chronic inflammatory processes of this nature act as a stimulus to monoclonal immunoglobulin production, in which case the pathogenic relationship with paraproteinaemia is reversed.

Lichen myxoedematosus

Lichen myxoedematosus (papular mucinosis) is a rare cutaneous disorder defined by a mucinous infiltrate in the upper dermis with fibroblast proliferation. Scleromyxoedema is a clinical variant characterized by diffuse skin thickening. The association of these conditions with paraproteinaemia is well documented, and the associated proteins are unusual in showing marked basicity and cathodal migration on electrophoresis (Lawrence, Tye & Liss, 1971). All but two of the abnormal proteins have belonged to the IgG class, and light-chain typing usually demonstrates lambda light chains (James *et al.*, 1967; Degos *et al.*, 1970; Lawrence *et al.*, 1971; Wells, Fudenberg & Epstein, 1972; Kitamura *et al.*, 1978). Although a few cases have shown limited numbers of abnormal plasma cells, bone marrow examination is more often normal. Myeloma was recorded by Perry, Montgomery & Stickney (1960) but this was not confirmed on follow-up (Wright *et al.*, 1976). Jablonska & Stachow (1972) reported a patient with IgG kappa myeloma and diffuse 'sclerodermatous' skin involvement who presumably had scleromyxoedema, although this possibility was not considered by the authors. The paraprotein was unusual in having kappa chains but this has since been recorded in another case of scleromyxoedema (Lai A Fat *et al.*, 1973). Immunochemical studies by Kitamura *et al.* (1978) indicated that the paraprotein in their case was in incomplete IgG molecule which lacked a significant antigenic portion of the Fd fragment. Similar proteins may be produced in heavy chain disease, but the benign prognosis of patients with lichen myxoedematous distinguishes the condition. Systemic involvement can occur in scleromyxoedema (McCuistion & Schoch, 1956; Dalton *et al.*, 1961), but is seldom a cause of significant morbidity.

Despite many attempts to assign a pathogenic role to the paraprotein, a satisfactory explanation for its association with cases of lichen myxoedematosus is lacking. Certain authors have suggested that the paraprotein should be considered as essential for the diagnosis (Hill, Crawford & Rogers, 1976) but there are several reports of clinically typical cases were no paraprotein has been detected (Rudner, Mehregan & Pinkus, 1966; Rowell, Waite & Scott, 1969; Tay & Khoo, 1970; Howsden, Herndon & Freeman, 1975; Jessen, Straight & Becker, 1978). Some cases have responded favourably to cytotoxic therapy (Feldman *et al.*, 1969, Wright *et al.*, 1976), which suggests that the paraprotein is of some aetiological importance, but the cases that responded were not always the ones with associated gammopathies (Howsden *et al.*, 1975; Jessen *et al.*, 1978). One case of lichen myxoedematosus showed spontaneous resolution despite the persistence of the paraprotein (Hardie *et al.*, 1979). The effect of plasmapheresis has yet to be determined, but an instructive parellel is provided by the association of pretibial myxoedema with circulating IgG globulins, weere plasmapheresis has proved beneficial (Dandona *et al.*, 1979). Again, however, this provides only circumstantial evidence for the pathogenicity of the abnormal globulins (Kriss, 1979).

Systemic capillary leak syndrome

This term describes a condition in which patients experience episodes of generalized angio-oedema. (Atkinson *et al.*, 1977). Attacks are cyclical, last between eighteen and thirty-six hours and are associated with a massive shift of fluid from the intra-vascular to the extra-vascular compartments. This process involves all plasma constituents and has been attributed to increased capillary permeability. Clinical effects include abdominal distention, dyspnoea, myalgia and hypotension. Attacks are self-limiting but death can occur from laryngeal involvement. Only six patients have been described with this syndrome of whom four exhibited a monoclonal IgG gammopathy (Clarkson *et al.*, 1960; Weinbren, 1963; Jacox, Waterhouse & Tobin, 1973; Atkinson *et al.*, 1977). One further patient had high levels of gamma-globulin but electrophoresis was not carried out (Preston, Rees & Spathis, 1962). The sixth patient had a normal electrophoretic pattern (Horwith *et al.*, 1967). Comprehensive studies have not revealed any abnormality that might influence capillary permeability in these patients. Prostaglandin and histamine metabolism, bradykinin, complement and coagulation systems were unaltered both during and between attacks (Atkinson *et al.*, 1977). Experimental attempts to relate the increased capillary permeability to the paraprotein were unsuccessful. A wide variety of therapeutic agents have proven ineffective, though plasmapheresis has yet to be used in this condition. Plasma volume expansion during the hypotensive phase may produce fluid overload and pulmonary oedema during the recovery phase.

This condition should be distinguished from the other variety of paraprotein associated angio-oedema which shows classical pathway activation and acquired deficiency of C_1 esterase inhibitor Russell Jones, 1980).

Necrobiotic xanthogranuloma

The term necrobiotic xanthogranuloma has been coined recently by Kossard & Winkelmann to describe a new clinico-pathological entity associated with paraproteinaemia (Kossard & Winkelmann, 1980). Patients present with multiple xanthomatous plaques and nodules affecting the face and neck, which show areas of atrophy and telangiectasia, and tend to ulcerate. All patients have periorbital involvement, and ocular damage may result from destruction and cicatricial scarring of the eyelids, iritis, uveitis, or subcutaneous involvement with proptosis.

The histological changes are diagnostic. Large granulomatous masses containing histiocytes, foam cells, Touton and foreign body giant cells are separated by areas of hyaline necrobiotic tissue. The inflammatory process always involves the reticular dermis, characteristically affects the subcutis as well and in approximately half the cases extends up to the epidermis. Occasionally vascular damage is a prominent feature and these cases are associated with severe cutaneous ulceration and a particularly poor prognosis. Case no. 2 reported by Kossard & Winkelmann (1980) required amputation of a limb, and a similar patient described by Headington as necrotising lipogranulomatosis died despite treatment with systemic steroids and cytotoxic therapy (Headington, 1979).

Investigation has revealed that most patients with necrobiotic xanthogranuloma have a paraprotein band on electrophoresis, or other evidence of a plasma cell dyscrasia. Of the eight patients reported by Kossard & Winkelmann (1980), six had a monoclonal IgG paraprotein, of which three were kappa and three lambda on immuno-electrophoresis. Rendall *et al.* (1977) reported two similar cases as atypical multicentric reticulohistiocytosis, both associated with monoclonal IgG paraproteins of which one was kappa and the other lambda. Investigations in the case reported by Headington revealed a monoclonal IgG cryoglobulin and evidence of classical pathway complement activation with low levels of C_1, C_2 and C_4 (Headington, 1979). Although this patient eventually succumbed, bone marrow examination did not revealed any evidence of myelomatosis. This, however, is not always the case. Three of the cases reported by Kossard & Winkelmann (1980) had myeloma or evidence of a lymphoproliferative process, so necrobiotic xanthogranuloma must be regarded as another cutaneous manifestation of malignant plasma cell dyscrasias.

Whether the condition can occur without paraproteinaemia is not certain. Cases fulfilling the clinical and histological criteria for this condition have been described under a variety of different titles, including atypical necrobiosis lipoidica (Muller & Winkelmann, 1966; Koczard, 1970), unusual forms of xanthoma disseminatum (Frank & Weidman, 1952; Maize, Ahmed & Provost, 1974), or xanthomatous granuloma (Frank, 1977). Not all these cases were associated with paraproteinaemia, but immuno-electrophoresis and bone marrow examination was not performed in all cases. Clearly the pathogenetic relationship between the paraprotein and the cutaneous lesions cannot be resolved until more is known about the aetiology of such disorders. Although most cases are normolipaemic, the lesions bear only a superficial resemblance to cases of diffuse plane xanthomatosis, and anti-lipoprotein activity was specifically excluded in one case (Headington, 1979). Immuno-peroxidase studies have shown that light chains are not specifically localized to any particular cell type (Kossard & Winkelmann, 1980) which excludes the possibility of local paraprotein production by the granuloma. IgG has however been demonstrated within histiocytic cells (Rendall *et al.*, 1977) and since macrophages carry FC receptors it was suggested that uptake of the anomalous immunoglobulin might act as a stimulus for proliferation of well-differentiated histiocytic cells. In any event the association of a monoclonal cryoglobulin, complement activation and tissue necrosis (Headington, 1979) suggests that the paraprotein may be causally implicated in this fascinating disorder.

Miscellaneous associations

There are many cutaneous syndromes which are recognized as markers of internal malignancy where the pathogenesis is unknown. Occasionally cases of myeloma occur in association with such syndromes, having been reported with erythema gyratum repens (Thivolet, Gallois & Perrot, 1970), dermatomyositis and acquired ichthyosis (Haynes & Curth, 1979). Generalized pruritus can be a presenting feature of myeloma in the absence of renal involvement (Erskine *et al.*, 1977), but may also occur with benign forms of monoclonal gammopathy (Zelicovici *et al.*, 1969). One syndrome which seems to be highly specific is the association of osteosclerotic myeloma with polyneuropathy, skin hyperpigmentation, hypertrichosis and oedema (Iwashita *et al.*, 1977). Although the great majority of patients have been Japanese, cases have also been reported in Europe (Crow, 1956; Waldenstrom *et al.*, 1978). The Japanese cases show a marked male predominance and may exhibit additional features such as hepatomegaly, white nails, clubbing, gynaecomastia and diabetes mellitus. Deposition of amyloid has been excluded as a cause of the polyneuropathy in these patients and the pathogenetic mechanism remains obscure. Although there is a high association with osteosclerotic myeloma, lytic bone lesions may coexist (Waldenstrom *et al.*, 1978) and a few cases have shown no evidence of myeloma other than a monoclonal gammopathy (Iwashita, Inoue & Naganatsu, 1971).

Angio-immunoblastic lymphadenopathy

Neoplastic M-component disorders arise in a variety of clinical settings, of which myeloma and Waldenstrom's macroglobulinaemia are the commonest. Other forms of lymphoproliferation have not been considered separately in this review as the associated cutaneous disorders depend upon the properties of the paraprotein rather than the mode of its production. An exception to this rule is angio-immunoblastic lymphadenopathy (AILD), a recently delineated lymphoproliferative disorder which resembles malignant lymphoma in its presentation and clinical cause, but shows a non-neoplastic type of lymph node histology with vascular proliferation and a mixed cellular infiltrate containing immunoblasts, plasma cells, lymphocytes and eosinophils (Frizzera, Moran & Rappaport, 1974). The condition is thought to represent an abnormal immune response to antigenic stimuli and there is good evidence implicating drugs such as phenytoin and penicillin or infective agents such as EB virus in the causation of this rare disorder (Purtilo, 1980). Because of polyclonal and monoclonal immunoglobulin production the condition is generally classified as a proliferative disorder involving B cells; yet cutaneous involvement occurs in almost half the cases (Bernstein, Soltani & Lorincz, 1979; Seehafer *et al.*, 1980). Occasionally the cutaneous lesions are nodular and show the same specific histology as seen on lymph node biopsy. Such lesions are therefore analogous to cutaneous plasma cell infiltrates in myelomatosis and carry a correspondingly poor prognosis. More often, however, a generalized eruption occurs as a transient phenomenon and such rashes are variously described as maculo-papular, petechial or erythrodermatous. The histology is correspondingly non-specific and gives little clue as to the aetiology, but such eruptions are not seen in pure varieties of plasma cell dyscrasia and are unlikely to be related to paraprotein production *per se*.

It could be argued that the cutaneous involvement in AILD is analogous to skin involvement seen in auto-immune diseases such as SLE, and that the paraproteinaemia represents a progression from polyclonal to monoclonal antibody formation, but this still does not explain whether the paraprotein and the cutaneous eruption are causally related or merely co-exist as manifestations of a more fundamental immunological derangement. Clearly the exact relationship between paraprotein production and association skin disorders of this nature cannot be satisfactory resolved until the precise mechanisms that induce B cells to proliferate are more clearly understood.

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Arylsulphatases in tumours

SIR, In a recent paper, Orfanos *et al.* (1979) mention that the ratio of arylsulphatase B to arylsulphatase A is much higher in homogenates from basal cell carcinomas than in those from normal epidermis or other epidermal lesions. Their assay is based on the pH curve of arylsulphatases in guinea-pig epidermis (Mier & van den Hurk, 1975): a curve with two optima, one at pH 4·6 caused by arylsulphatase A, the other at pH 5·9 due to arylsulphatase B. This identification is supported by older literature using nitrocatechol sulphate as a substrate. However, several authors were not able to differentiate sulphatase A from B by pH differences with 4-methylumbelliferyl sulphate: the optima were 5·5 for both enzymes in liver (Hultberg, 1977), 5·5 (Stevens, 1974) or 5·7 and 5·7–6·0 (Mercelis, Van Elsen & Leroy, 1979) in fibroblasts, 5·7 and 5·4 in brain (Harinath & Robins, 1971) and 4·8 and 5·1 in leukocytes (Christomanou & Sandhoff, 1977). In our experiments with sulphatases from basal cell carcinomas separated by ion exchange chromatography on DEAE cellulose the optima were respectively 5·0 for A and 5·2 for B.

The sulphatase A fractions were inhibited specifically by $AgNO_3$ (Hultberg, 1975; Mercelis *et al.*, 1979). The total activities we measured were 5–20 times higher than those mentioned by Orfanos *et al.* (1979) so that we do not think that our sulphatases are identical to theirs.

Two other circumstances suggest that the assays of Mier & van den Hurk (1976) and of Orfanos *et al.* (1979) were not done under optimal conditions:

(1) The supernatant of a centrifuged homogenate may not contain the full enzymatic activity present in the lysosomes (De Bersaques, 1980).

(2) Their assays were done with substrate concentrations that are probably well below the Km of the enzymes, which are given in the literature (Fluharty *et al.*, 1974; Harinath & Robins, 1971; Mercelis *et al.*, 1979; Rinderknecht *et al.*, 1970; Stevens, 1974; Stevens *et al.*, 1975) as between 8 and 25 mM for sulphatase A and 2·1 to 12 mM for sulphatase B. Our own Km's are respectively 3·2 mM for A and 7·7 mM for B.

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Reply

SIR, Synthetic substrates such as 4-methylumbelliferyl sulphate are hydrolysed by a complex group of enzymes collectively termed 'arylsulphatases'. Some years ago we examined the arylsulphatase activity of extracts of normal and diseased human epidermis, and showed that at pH 4·6 and a substrate concentration of 0·5 mmol/l the rate of hydrolysis was invariably greater than at pH 5·9 and a substrate concentration of 2 mmol/l (Mier & van den Hurk, 1976). More recently we reported that this ratio was reversed in extracts of basal cell carcinoma (Orfanos *et al.*, 1979). In both papers we assumed that the activities under the two different sets of conditions could be ascribed to arylsulphatases A and B respectively.

We agree with de Bersaques that this assumption seems untenable in the light of his present observations and other recent data. Nevertheless, our actual findings remain valid; we are certainly able to distinguish basal cell carcinomas from non-malignant epidermis in the manner described. What, then, is the explanation?

Many possibilities can be envisaged. For example, natural arylsulphatase substrates (such as sulphated glycosaminoglycans) present in the epidermal extract will competitively inhibit the hydrolysis of synthetic substrates, the inhibition being greater at lower concentrations of the latter. Thus the altered ratio in basal cell carcinomas might simply reflect the presence of abnormally large amounts of an endogenous sulphate in the malignant tissue. Such an effect would, of course, disappear following ion-exchange chromatography of the tissue extracts. Further experimental work in this direction might well be rewarding.

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Book Reviews

Hormone Therapy of Acne. Clinical and Experimental Principles. DORIS FANTA (1980) Wien-New York: Springer-Verlag. Pp. 91, Illustr. 25. Soft cover. Price DM 34,00, US $20.10.

This is the updated English translation of 'Akne, Klinische und experimentelle Grundlagen zur Hormontherapie' which was published in 1978. The author, who works in Vienna, has concisely reviewed the world literature on the hormonal regulation of sebum production and its role in acne therapy, and has added brief accounts of her work in this field. She has produced a readable and reasonably well-balanced overview of the subject which will be of great value to those who are new to this complex subject. More experienced workers will benefit mainly from the many references to the German literature, which is sometimes shamefully neglected by British and American authors. The work on local oestrogen application cited by Dr Fanta, for example, is rarely mentioned in the English literature.

The translation was very good, apart from a few neologisms such as 'destroyance' (p. 24), but a printing error on p. 50 has led to the dangerous suggestion that 20 mg ethinyl oestradiol daily is a very low dose. Overall though, this is a very useful little book.

J.L.BURTON

Manual of Skin Diseases. GORDON C. SAUER (1980) London: 4th Edition. Pp 371. 84 col. illus. Price: £22.50. Published by Lippincott, distributed by Harper & Row Ltd.

This book is intended to present to medical students and general practitioners material that they must know for the diagnosis and treatment of patients with common skin diseases.

It is written in a colloquial style with dogmatic advice, some of which might not find favour with British Dermatologists. For example amongst six possible treatments for warts are listed Synalar cream with polythene occlusion and vitamin A 50,000–100,000 units a day for three months 'for the resistant case and for the one who says "Doctor, aren't there any pills I can take for these warts" '. Topical neomycin is recommended for the treatment of stasis ulcers without any warning of the risk of sensitization.

I found the order of the chapters a little illogical. There is an introductory chapter on the structure of the skin and then after further chapters on laboratory procedures and tests, diagnosis, history taking, therapy, cosmetics for the dermatologist, there is a chapter entitled 'Dermatological Allergy' which covers endogenous and exogenous eczemas as well as drug eruptions. This is followed by a chapter on 'Dermatological Immunology' before the book reverts to clinical dermatology. Similarly I found it a little odd that the chapter on genodermatoses was separated from the chapter on paediatric dermatology by a chapter on tumours of the skin.

The book is (not surprisingly) American orientated and some of the statements made should be seen in this light, e.g. 'two of the commonest contact dermatoses seen in the doctor's office are poison ivy dermatitis and hand dermatitis'.

The price of the book has been kept down by sponsorship by several drug companies for the colour plates, which are, in general, of a high standard, although some appear quite as good in duplicate black and white photographs. Possibly as a result of this sponsorship most drugs and topical agents are mentioned by proprietary names, a factor which may cause slight confusion.

This book gives concise guidance on diagnosis of most common skin conditions and I would recommend it for this, but I have reservations about some of the therapy suggested.

P.M.HUDSON

Entomology in Human and Animal Health. 7th edn. R.F.HARWOOD AND M.T.JAMES (1979) New York, Macmillan. Published by Baillière Tindall, 35 Red Lion Square, London WC1R 4SG. Pp. viii, 548. Price £18.

The first edition of this well known work was published in 1915 with the title of 'Medical and Veterinary Entomology'; the author was W.B.Herms. In 1961 and 1969 Maurice James, Professor of Entomology, Washington State University, Pullman, was co-author with Dr Herms of the fourth and fifth editions. For the sixth edition in 1969 Dr James was joined by Dr Robert Harwood who has succeeded him in the Washington State Chair of Entomology. In many ways this is a new book rather than a new edition for it has been almost completely rewritten with a more international approach and with emphasis on modern methods for the control of arthropods.

The earlier chapters provide a clear summary of the structure and classification of arthropods and of relevant aspects of their physiology and epidemiology. There follow systematic accounts of the main classes of arthropods with a detailed discussion of those species which affect human or animal health. The references cover recent publications in the international literature, but also draw attention to important and comprehensive monographs, where further information concerning important species can be sought. A final chapter entitled 'Venoms, Defense Secretions, and Allergens of Arthropods' usefully brings together many facts of special concern to dermatologists which are mentioned only briefly in the earlier chapters.

Several studies in different countries, temperate and tropical, have shown that, with disconcerting frequency, acute or chronic prurigos and a variety of ill-defined pruritic syndromes can be proved by diligent investigation by entomologically well-informed physicians and veterinary surgeons to be caused by the activities of unsuspected arthropods in allergically sensitive subjects. The dermatologist requires a text which relates clinical syndromes to possible exposure to arthropods in a variety of environments and climates and which will also provide a comprehensive review of the large literature on immunological responses to arthropods. The present volume, although its approach is much more general and it does not completely satisfy the need, can be strongly recommended to dermatologists as an excellent work which will solve many practical problems in the clinic and will guide the interested reader through the forbiddingly massive literature of applied entomology.

ARTHUR ROOK

Paraneoplasia: Biological Signals in the Diagnosis of Cancer. JAN G.WALDENSTRÖM (1978) Chichester, John Wiley and Sons. Pp. 190, 6 coloured illustrations. Price £16.10.

This book is the product of a distinguished academic general physician and biochemist; the result of many years of personal involvement in the problems of paraneoplastic phenomena and of wide reading. It would be unreasonable to expect, and the reader will not find, complete coverage of the dermatological literature in this perplexing field. Nor is this a concise standard text on paraneoplasia. What the reader will discover is an excellent brief introduction followed by a series of chapters on cutaneous, neurological, haematological, vascular, renal and skeletal aspects of this subject as they present clinically. The second part of the book is concerned with biochemical abnormalities which may result from neoplasia and with the fundamental mechanisms involved. There are many cases histories from Prof. Waldenström's personal experience and from the literature, and if occasionally the author strays somewhat from the strictest interpretation of his brief there is compensation in his great enthusiasm for the subject which shines through every chapter. The book reads like an extended tutorial, set in historical perspective, meandering a little but never losing sight of the problem. Those of us with a dermatological interest in this subject will find this a stimulating book, and all should heed Prof. Waldenström's call to be rigorously critical about accepting causal associations between clinical features and co-existing neoplasms.

S.O.B.ROBERTS

Diseases of the External Ear. An Otologic-Dermatologic Manual. B.H.SENTURIA, M.D. MARCUS AND F.E.LUCANTE (1980) 2nd Edition New York; Grune and Stratton. London: Academic Press. Pp. 204, Illust. 263 (mostly colour). Price £16.80.

The abrupt change in the nature of the epithelial lining of the cartilaginous and osseous parts of the external auditory canal makes as good a frontier as any of the interests of the dermatologist; the auricle and its surrounding area are his main interest, the external part of the canal common ground; beyond that a labyrinth full of mysteries. But for the otorhinogologist the whole ear is his oyster and it is his specialty that has provided most of the research into the inflammatory conditions of the auditory canal. This difficult and complex subject is set out with enviable clarity, especially the role of primary and secondary fungal infections. The co-authorship of Dr Marcus ensures that dermatological conditions are fully represented and, indeed, it is difficult to find any omission in this succinct account. A particularly useful feature of the book is the inclusion of chapters on methods of examination, the basis of therapy and a discussion of the importance of choosing the correct vehicles. The high standard of the well chosen line drawings and colour illustrations enhances the text, which is suffused throughout with commonsense, a commitment to simple treatment whenever possible and an evident regard for the well-being of the patient as a whole. It would be easy for the fastidious to point to obvious differences between American and British usage—the elliptical biopsy, for instance, is not mentioned and Mohs' technique for malignant epithelial tumours is given pride of place. Contact allergic dermatitis is given its full importance but the need for careful and authoritative patch test techniques receives rather cursory attention. There are a few, unimportant, typographical errors but the standard of printing and the excellent style in which the book is written enable it to be read with pleasure as well as with profit. A worthy successor to Professor Senturia's original classic of some 23 years ago, it must find a place in every dermatologist's working library.

D.S.WILKINSON

Basic and Diagnostic Aspects of Cutaneous T Cell Lymphomas. E.M. VAN DER LOO (1980) Pijnacker: Dutch Effiency Bureau. Pp. 160.

The contribution made to the study of cutaneous lymphomas by the departments of pathology and dermatology of the University of Leiden is well recognized by all workers in the field. This small book is a collection of seven papers on T Cell lymphomas of the skin together with an introduction and a summary, and forms Dr van der Loo's doctoral thesis.

The work is described as a morphological, cytochemical and immunological study, and different chapters deal with different investigative techniques used in the diagnosis and assessment of mycosis fungoides, Sèzary syndrome and related disorders.

The accurate measurement of nuclear contour of lymphoid cells on electron micrographs, the characterization of cerebriform mononuclear cells in the blood of healthy individuals and the use of cytophotometry in the study of T cell lymphomas are some of the subjects covered. There is very little to criticize in this book, but one must be aware of the limitations of some of the techniques. For instance, it is stated that the DNA content of cerebriform mononuclear cells (measured by cytophotometry) can be used as a diagnostic criterion to diagnose early mycosis fungoides, although it is known that in lymphomatoid papulosis, a disorder with a benign course in the great majority of cases, the DNA histograms are markedly abnormal. Not everyone would agree with the statement that 'the premycotic stage (of mycosis fungoides) is clinically as well as histologically non-specific'. These are minor carps, however, and although most of the chapters have already been published separately, it is most useful to have these papers collected in one volume. The book can be heartily recommended to everyone working or interested in the field of cutaneous lymphomas.

NEIL SMITH

Cytodiagnosis in Dermatology. V.Ruocco (1980) Naples: Cooperativa Libraria Universitaria. Published by Cooperativa Libraria Universitaria Via Leonardo Bianchi 5, Naples, Italy. Pp. xiii + 145.

This well-produced small book deals with the applications of cytological diagnosis to a wide range of disorders affecting the skin. The useful first sections of the book consist of a description of the procedure for taking smears and details of various staining techniques. The author then discusses the cytology of smears from normal skin followed by sections on a wide range of different cutaneous disorders, both inflammatory and neoplastic.

Professor Ruocco is obviously an enthusiast and the value of cytological diagnosis in certain conditions such as herpes simplex is undisputed, but one seriously questions how useful the technique is likely to be when applied to the wide range of different disorders dealt with in this book. Is the examination of skin smears really likely to help with the diagnosis of cutaneous lymphomas? Although, when referring to malignant melanoma, the author admits he likes to be 'exceedingly prudent' in the application of cytodiagnostic techniques, the inclusion of a chapter on this subject implies that he considers the procedure of value. It seems difficult to justify this view particularly if a biopsy for histological examination is taken at the same time as a smear for cytodiagnosis.

It would be interesting to have some indication of the success rate of cytology as a diagnostic tool when compared with more orthodox procedures such as biopsy and histological examination. A further minor criticism is that the colour illustrations on the right-hand pages of the book frequently do not correspond to the conditions discussed in the text, so that the section on Grover's disease is found opposite photographs of cells from naevocytic naevi and keratoacanthoma.

Although the illustrations are of good quality and Professor Ruocco provides us with an interesting bedtime read, it is doubtful whether many dermatologists will find much of lasting practical value in the pages of this book.

Neil Smith

Photochemotherapy. Information for Doctors and Patients. Gerhard Weber (1980) London: YB Medical Publishers Ltd. Pp 106. 4 Col. plates. 68 B/W figs. Price £8.75.

This small monograph starts with a historical review of the psoralens and how they came to be used in the treatment of skin disease, and in particular the development during the 1970s of their topical application along with long wavelength (UV-A) radiation, ultimately leading to their oral administration and the development of present-day psoralen photochemotherapy. This is followed by a discussion of the irradiation equipment required, the organization of a therapy unit and the results of the author's experience, particularly in the treatment of psoriasis, but also of other dermatoses. A protagonist of the topical application of the psoralens in preference to oral administration, the author may be somewhat out of step with current opinion but this record of his experience is useful and valuable. Any treatment is liable to be modified with the passage of time and this makes the writing of such a monograph difficult in that it runs the risk of being left somewhat behind as views change.

Professor Weber's publication will be read, therefore, for its value as a record of clinical experience rather than as an up-to-date practical 1980 guide for those involved in running and developing a photochemotherapy unit. This in no way detracts from a recognition of the part played by its author in the development of psoralen photochemotherapy. In trying, however, to produce a text suitable for both clinicians and patients, the author has probably attempted the impossible and many may feel that this sort of publication is not suitable for the majority of lay patients.

W.Frain-Bell

British Journal of Dermatology (1981) 104, 227.

Report on the Meeting of the International Committee of Dermatology

The International Committee of Dermatology met at the Hotel New Otani in Tokyo, April 19–20 1979 to examine the site and to discuss the organization and scientific aspects of the XVI International Congress of Dermatology. The committee members were highly impressed by the facilities demonstrated to them and the advanced planning and preliminary organization already performed by the Japanese organization committee. The definite date for the International Congress was confirmed to be Sunday May 23–Friday May 28 1982. The decision was made that abstracts of all papers presented at the XVI International Congress as well as Proceedings of this Congress will be published. Abstracts of the papers to be given should be ready before the Congress at a time determined by the Organization Committee; the Proceedings volume was agreed to include full length reports of Special Lectures; of the papers on Advances in Dermatology, papers presented in the Symposia and Case Presentations I and II. Summaries of Workshops and Posters will also be published as will be Free Communications either in abstract or full length, the latter at the discretion of the Editorial Committee. English will be the publication language. Summaries of Courses, Informal Discussions and Exhibits will not be published.

Since there have been some difficulties in the continuous communication between the ICD and the national societies, mostly due to the fact that the officers of most of the national societies change every year, the ICD decided to pass a resolution to the effect that each national society should be requested, through the Secretary-General, to form a standing committee on international matters to serve as a liaison with the ICD and the Organizing Committee of the International Congress. In order to make information available on the actions and purpose of the International League of Dermatological Societies and the International Committee of Dermatology, the Secretary-General has prepared a manual of the International League of Dermatological Societies which is to be sent to the presidents, secretaries and delegates of the national societies. The manual will also be handed out to the participants of the XVI International Congress. A revision of the bylaws of the ICD is also planned and work so far done by an *ad hoc* committee consisting of Professors Cabré, Civatte, Thyresson, Winkelmann and Wolff was discussed. A proposal for the new bylaws is to be sent to the member societies at least three months before the next meeting of the Assembly of Delegates in Tokyo, 1982.

A committee for extracongressional matters was created which is to present thoughts on international actions for mutual help in dermatology under the sponsorship of the ICD. This committee consists of Professors Winkelmann (chairman), Civatte, Dominguez-Soto, Seiji and Wilkinson.

Finally, new members of the ICD will have to be elected at the International Congress in Tokyo. Present members of the ICD who will be replaced in 1982 according to the bylaws are Professors Baer, Braun-Falco, Cordero, Jablonska and Padilha-Goncalves. The Secretory-General was requested to write to the member societies and ask for proposals of ten names for the vacancies with only two names to be named from any one country.

One of the last major items brought up and discussed at this meeting was a report by Professor Civatte on the criticism which has been voiced by some French newspapers concerning case presentations at medical meetings. The ICD unanimously accepted a resolution put forward by Dr Wilkinson which reads as follows:

'*Presentation of patients at dermatological meetings.* Apart from the educational aspect, close scrutiny of the skin is a necessary part of clinical diagnosis in dermatology. Though modern technology has evolved alternative methods of presenting dermatological case materials for discussion, there are limitations to all audiovisual techniques in current use. At the present time, therefore, it may sometimes be necessary and even advantageous to the patient that he or she is presented personally at meetings of dermatologists for accuracy of diagnosis or the establishment of the best treatment. We regard this practice as acceptable, provided that the patient gives his informed consent and that the presentation is carried out under conditions of adequate space and time and with dignity and humanity on the part of all concerned.'

Professor NILS THYRESSON
Secretary-General, ICD

British Journal of Dermatology (1981) **104**, 229.

News and Notices

Netherlands Association of Dermatology and Venereology

The next meeting of the Netherlands Association of Dermatology and Venereology will be held in the Dermatological Department of Leiden University on 21 February 1981.

Further information may be obtained from Dr S.M.C.Molhuysen-van der Walle, Guido Gezellestraat 6, 1077 WP Amsterdam, The Netherlands.

European Congress of Dermocosmetology

The 1st European Congress of Dermocosmetology (14th European Conference) will be held 19–21 May 1981 in Lyon, France. Topics include: structure and function of the stratum corneum, skin culture techniques, free communications and the exploration of the surface of the skin.

Further information may be obtained from: Centre European de Dermocosmetologie, BP 10 Lyon Mouche, 69342 Lyon Cedex 2, France.

Alfred Marchionini Prize 1982

On the occasion of the XVI International Congress of Dermatology in Tokyo, the Alfred Marchionini Prize founded in 1966 will again be awarded.

For the Alfred Marchionini Research Grant—the second stage of this prize (which is to be understood as an international scholarship)—proposals are invited before July 1 1981, to the chairman of the Board of the Alfred Marchionini Foundation, Professor Dr Th. Nasemann, Director of the Dermatological Hospital of the University of Hamburg, D-2000 Hamburg 20, Martinistrasse 52, or to the Secretariat of the Alfred Marchionini Foundation, D-2057 Reinbeck, Danziger Strasse 5.

According to article 8 of the statutes of the Alfred Marchionini Foundation, medical directors of hospitals, research or other scientific institutes, are entitled to submit proposals.

Young scientists in the special fields 'Medicine' (preferably dermatologists), 'Biochemistry' and 'Biopharmacy' of all nationalities, who deserve encouragement, are to be considered as candidates for the award of the Alfred Marchionini Prize—stage 2.

In addition to the merit rating, the interest in international human relations in accordance with the intentions of Alfred Marchionini, as well as the scientific work of the candidate that must be of particular importance to international dermatology, will be taken into account in making the award.

Proposals must include a detailed appreciation of the candidate as well as reasonable arguments and corresponding documents.

The Alfred Marchionini Prize 1982 (research grant) is endowed with DM 20,000.

APPOINTMENTS

Glasgow Royal Infirmary

Dr Nick Simpson has been appointed Consultant Dermatologist at Glasgow Royal Infirmary. Dr Simpson qualified MB BCh (Wales) in 1971 and MRCP (UK) in 1975. After a registrar post at the Western Infirmary in Glasgow under the late Professor J. A. Milne, he was appointed Senior Registrar and honorary tutor in Dermatology at the General Infirmary at Leeds in 1977.

His main research has concerned the physiology and pathology of sebaceous glands, on which he has published several papers. His other research interests lie in cutaneous reactions to drugs and the involvement of the skin in renal disease.

Corrigendum

The title of the paper by E.S.Falk and R.Bolle in the October 1980 issue, p. 367, should have been '*In vivo* demonstration of specific immunological hypersensitivity to scabies mite' rather than '*In vitro* . . . .' (Apologies, ed.)

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr R.H.Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldly names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a,) b. All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of journal in full; (5) volume; (6) number of the first page of the article. *Thus*: CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to books and monographs should include: (1) author(s) or editor(s); (2) year of publication; (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*. BEARE, J.M. & WILSON JONES, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 104, Number 2, February 1981

CONTENTS PAGE

Clinical and Laboratory Investigations

Human Dermal Microvasculature: I. Its segmental differentiation. Light and EM Study 117
J.C.HIGGINS AND R.A.J.EADY

Skin lesions in angioimmunoblastic lymphadenopathy: histological and immunological studies 131
M.G.BERNENGO, L.LEVI AND G.ZINA

T Lymphocytes and mononuclear phagocytes in the skin infiltrate of systemic and discoid lupus erythematosus and Jessner's lymphocytic infiltrate 141
Y.T.KONTTINEN, S.REITAMO, A.RANKI AND M.SEGERBERG-KONTTINEN

Acid glycosidases in basal cell epithelioma and in seborrhoeic keratosis 147
J. DE BERSAQUES

Follicular psoriasis 153
L.STANKLER AND S.W.B.EWEN

Repeated patch testing in penicillin allergy 157
D.P.BRUYNZEEL AND W.G.VAN KETEL.

The influence of ultraviolet radiation on allergic contact dermatitis in the guinea-pig. I. UVB radiation 161
WARWICK L.MORISON, JOHN A.PARRISH, MICHAEL E.WOEHLER AND KURT J.BLOCH

The influence of ultraviolet radiation on allergic contact dermatitis in the guinea-pig. II. Psoralen/UVA radiation 165
WARWICK L.MORISON, JOHN A.PARRISH, MICHAEL E.WOEHLER AND KURT J.BLOCH

Isolation and biochemical characterization of tyrosinase-rich GERL and coated vesicle in melanin synthesizing cells 169
GENJI IMOKAWA AND YUTAKA MISHIMA

Pharmacology and Treatment

Halogenation and topical corticosteroids: a comparison between the 17-butyrate esters of hydrocortisone and clobetasone in ointment bases 179
C.F.ALLENBY AND C.G.SPARKES.

Factitious urticaria (dermographism): treatment by cimetidine and chlorpheniramine in a randomized double-blind study 185
SURRINDER KAUR, MALCOLM GREAVES AND NAHID EFTEKHARI

Topical treatment of recurrent herpes simplex (HS) and post-herpetic erythema multiforme (PHEM) with low concentrations of zinc sulphate solution 191
ISSER BRODY

Case reports

Report of an infant with GM_1 gangliosidosis type I and extensive and unusual mongolian spots 195
MARC WEISSBLUTH, NANCY B.ESTERLY AND WILLIAM A.CARO

Dapsone and severe hypoalbuminaemia in dermatitis herpetiformis 201
R.E.COWAN AND J.T.WRIGHT

Brief Communications

Ketotifen and terbutaline in urticaria 205
ERIC M.SAIHAN

Serum immunoglobulins in lichen planus 207
JOHN M.MAHOOD

Review Article

Cutaneous manifestations of paraproteinaemia—part II 209
R.RUSSELL JONES

Correspondence 221

Book Reviews 223

International Committee of Dermatology meeting 227

News and Notices 229

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This is a concise nursing text which classifies skin disorders and details the nursing therapy required. It covers all aspects of dermatological illnesses, from the physical to the psychosomatic, introducing both common and rare skin complaints and emphasises the importance of nursing management. Nurse-patient and doctor-patient relationships are stressed as not only complementary to but inseparable from scientific dermatology.

The text is abundantly illustrated with black and white and colour photographs and the authors' highly readable and informative text will make this book invaluable to all those involved with the care of patients suffering from skin disorders.

Emergency Procedures and First Aid for Nurses

Muriel Skeet
July '81. 256 pages, 114 illustrations.
Paper, about £4.50

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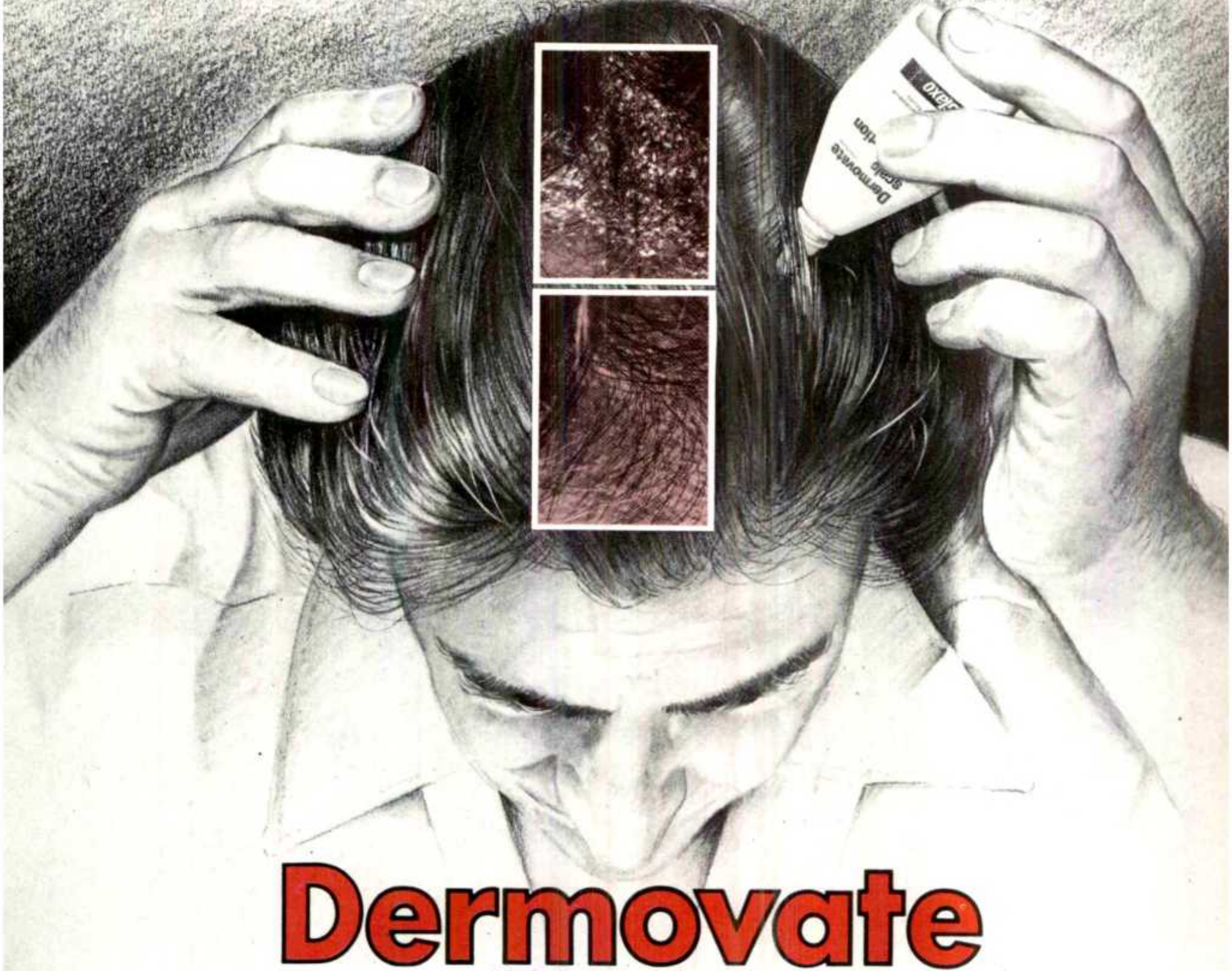

Dermovate

(clobetasol propionate)

Scalp Application

Prescribing information

Uses

Psoriasis and recalcitrant eczemas of the scalp. Clobetasol propionate is a highly-active topical corticosteroid which is indicated for use in short courses for conditions which do not respond satisfactorily to less active steroids.

Dosage and administration

Apply sparingly to the scalp night and morning until improvement occurs. As with other highly-active topical steroid preparations, therapy should be discontinued when control is achieved. Repeated short courses of Dermovate Scalp Application may be used to control exacerbations. If continuous steroid treatment is necessary, a less potent preparation should be used.

Contra-indications

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Precautions

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Development of secondary infection requires withdrawal of topical corticosteroid therapy and institution of appropriate systemic antimicrobial therapy.

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In general, topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

Dermovate preparations are usually well tolerated, but if signs of hypersensitivity appear, application should be stopped immediately. As with other topical corticosteroids, local atrophy may result from prolonged treatment, particularly if occlusive dressings are used. When extensive areas are treated, sufficient systemic absorption may occur to produce the features of hypercorticism, especially in infants and children. This effect is more likely to occur after prolonged treatment, particularly if occlusive dressings are used. In rare instances, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Presentation

Plastic squeeze bottle with elongated nozzle containing 25 or 100ml

Basic NHS cost (exclusive of VAT)

25ml bottle £2·72

100ml bottle £9·65

Product Licence number

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Glaxo

Leaders in topical steroid therapy

Further information on Dermovate (trade mark) Scalp Application is available from

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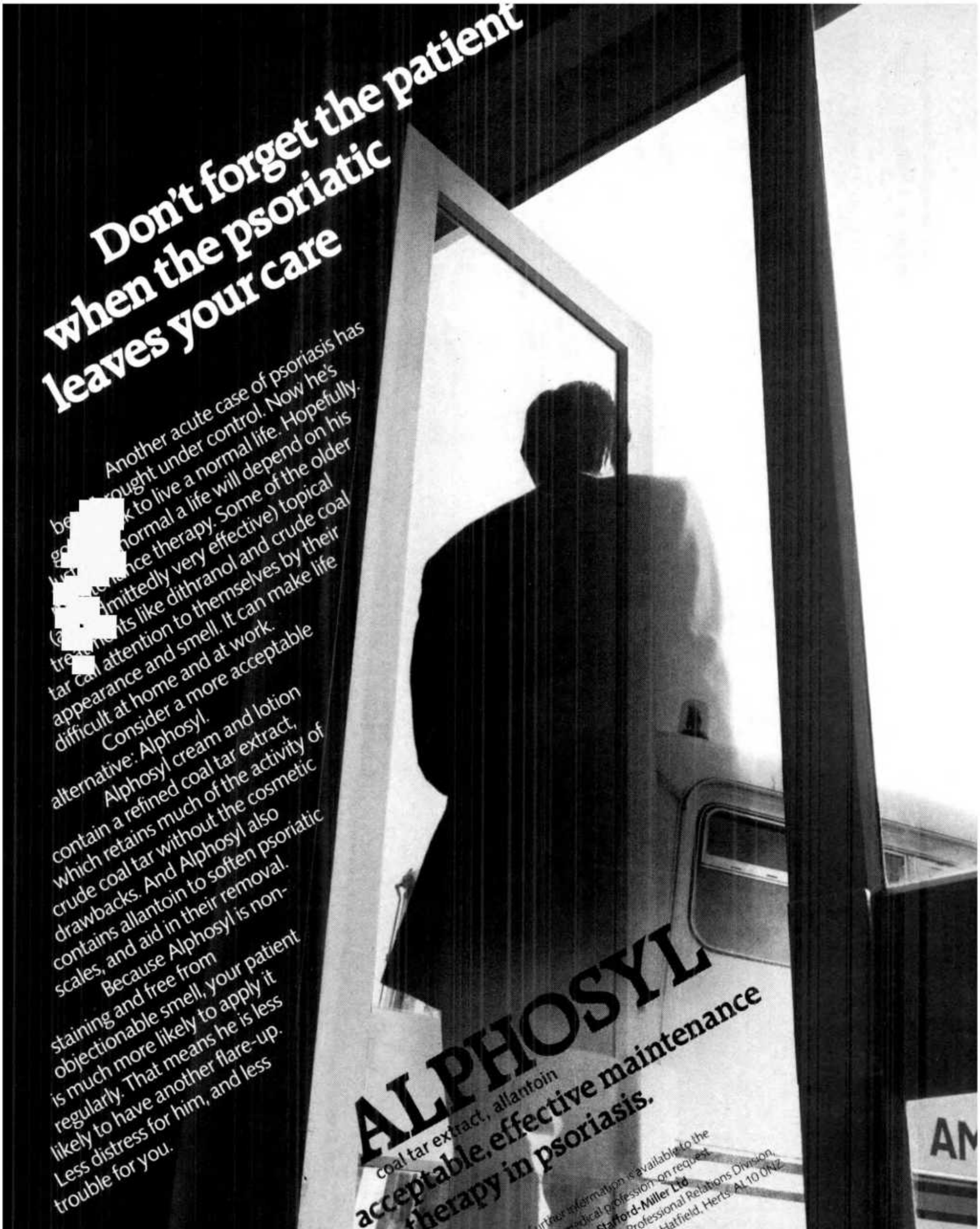

Don't forget the patient when the psoriatic leaves your care

Another acute case of psoriasis has be[illegible]rought under control. Now he's go[illegible]k to live a normal life. Hopefully. [illegible] normal a life will depend on his [illegible]nce therapy. Some of the older ([illegible]mittedly very effective) topical tre[illegible]ts like dithranol and crude coal tar ca[illegible] attention to themselves by their appearance and smell. It can make life difficult at home and at work.

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coal tar extract, allantoin

acceptable, effective maintenance therapy in psoriasis.

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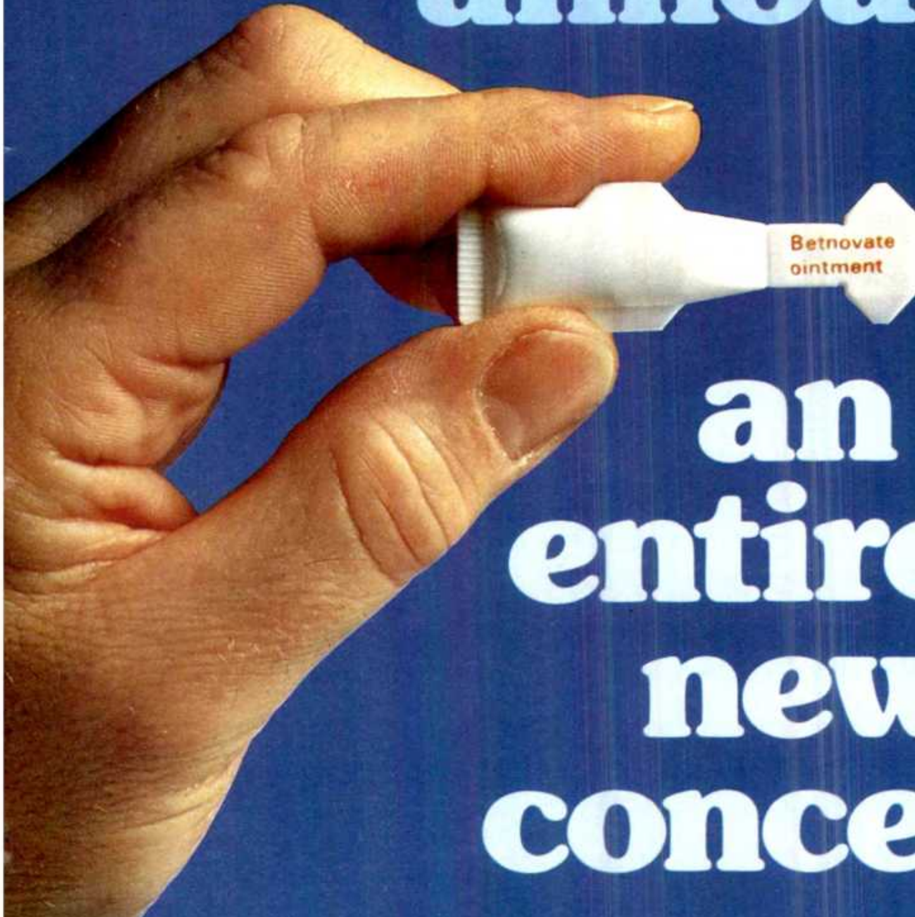
Presentation LOTION AND CREAM: Containing allantoin 2%, refined alcoholic extract of coal tar 5%, in a greaseless vanishing-cream base. The lotion is a light, free-flowing emulsion and the cream is smooth and homogeneous, light tan in colour. **Uses** Dermatological stimulant for the treatment of psoriasis. **Dosage and Administration** Apply liberally two to four times daily, massage well into the affected areas. Occlusive dressings are unnecessary. **Contra-indications and Warnings, etc.** For external use only. Discontinue if irritation occurs or in cases of sensitivity to coal tar. **Pharmaceutical Precautions** Do not refrigerate. **Legal Category** No legal restrictions on sale or supply. **Package Quantities** ALPHOSYL LOTION: Bottles of 250ml. ALPHOSYL CREAM: Tube of 60 **Further Information** Alphosyl Lotion is the basic treatment for psoriasis of the body and scalp. Alphosyl Cream is designed especially for lubrication in the intertrigenous areas. **Product Licence N**

Glaxo announce



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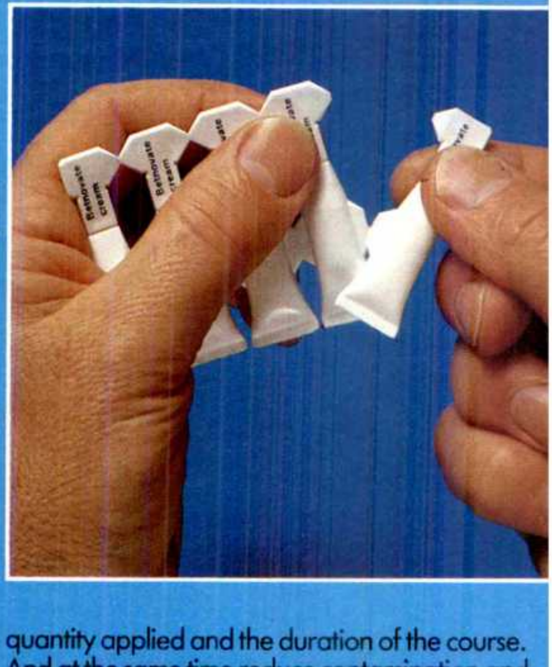

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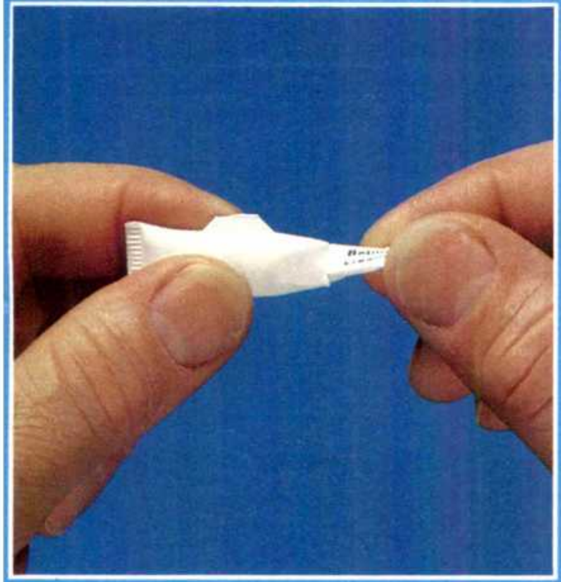
Prescribing information

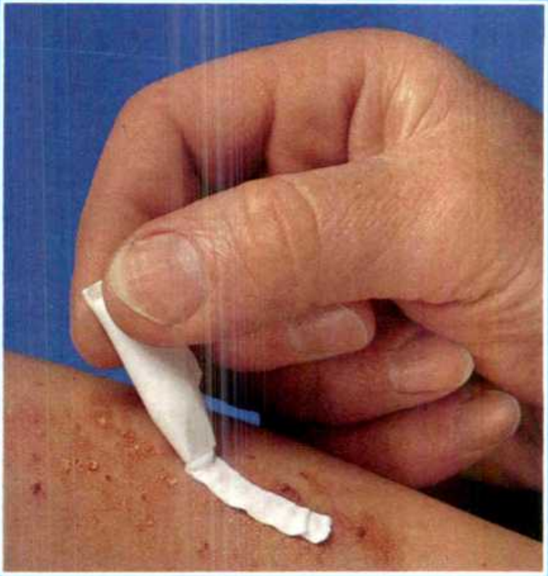
Dosage and administration
Sufficient cream or ointment should be applied to leave the affected area looking glossy. This should be applied up to three times daily depending on the circumstances of the case. However, with Dermovate once or twice daily application is sufficient.

Side effects
General:
With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also, prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism. This is more likely to occur in infants and children, and with occlusion. In infants the napkin may act as an occlusive dressing. Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.
In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.
Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Precautions
Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit

A new measure of control in topical steroid therapy





In comparison with tube presentations:

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Glaxo Leadership and experience in topical steroid therapy

atrophic changes after prolonged treatment with potent topical corticosteroids.

Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy.

With all corticosteroids, prolonged application to the face is undesirable.

Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications

Bacterial, fungal or viral diseases of the skin. Dermovate and Betnovate are also contra-indicated for rosacea, acne and peri-oral dermatitis. Hypersensitivity to the preparation.

Basic NHS cost (exclusive of VAT)	**Product Licence numbers**
Betnovate Cream/Ointment Flexules 20 × 1 gram £3·62	4/5121, 4/5124
Dermovate Cream/Ointment Flexules 20 × 1 gram £4·22	4/0219, 4/0220
Eumovate Cream/Ointment Flexules 20 × 1 gram £3·91	4/0233, 4/0254
Efcortelan Cream/Ointment 1% Flexules 20 × 1 gram £3·19	4/5081, 4/5086

Betnovate, Dermovate, Eumovate, Efcortelan and Flexules are trade marks. Further information is available from:
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Clinical and Experimental Dermatology

Edited by Martin M. Black
Institute of Dermatology, St. John's Hospital for Diseases of the Skin, London, England

This journal publishes original articles covering all aspects of the normal biology of the skin and clinical and experimental dermatology. Regular features include the full text of bi-annual orations by internationally renowned speakers on topics of major dermatological interest and Society clinical proceedings. Invited review articles, book reviews and correspondence are published occasionally. *Clinical and Experimental Dermatology* was formerly titled *Transactions of the St. John's Hospital Dermatological Society* and, although the official organ of the hospital, attracts contributions from all countries and its circulation is equally international.

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Why mess about with topical antifungals ...when oral Grisovin can be working from within?

For fungal infections, topical therapy is often both messy and inconvenient – and is unlikely to be as completely effective as oral Grisovin.

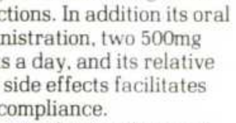
Grisovin is carried in the bloodstream to the basal layer and gets right to the root of ringworm infections. It protects the newly formed keratin cells from infection and as they move outwards, the fungus is expelled.

During the past 20 years, Grisovin has proved itself in clinical use to be the more effective therapy especially in long established ringworm infections. In addition its oral administration, two 500mg tablets a day, and its relative lack of side effects facilitates patient compliance.

Why mess about with topical antifungals when Grisovin gets right to the root of the problem?

GRISOVIN

griseofulvin



THE INSIDE STORY ON FUNGAL INFECTION

Prescribing information

Dosage and administration

Doses should be taken after meals for maximum absorption.

Adults: normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily.

Children: usually 10mg/kg (5mg/lb) bodyweight daily in divided doses.

Duration of treatment: for hair or skin at least four weeks' treatment is required, whereas finger or toe nails may need six to twelve months' treatment. Continue for at least two weeks after all signs of infection have disappeared.

Contra-indications

Porphyria or severe liver disease.

Griseofulvin may cause deterioration in some patients with liver disease and liver function should be monitored in such conditions.

Precautions

Griseofulvin may decrease the effect of coumarin anticoagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established, but use during pregnancy is best avoided.

Side effects

Headache, and gastric discomfort sometimes occur, but usually disappear as treatment continues. Rarely, urticarial reactions and erythematous rashes have been reported.

Further information

Grisovin is not effective in infections caused by *Candida albicans* (monilia), Aspergilli, *Malassezia furfur (Pityriasis versicolor)* and Nocardia species.

Basic NHS cost	**Product Licence numbers**
(exclusive of VAT) £9.80	
Bottle of 100 tablets (500mg)	125mg 4/5060 500mg 4/5061

Glaxo

Full prescribing information on Grisovin (trade mark) is available from:

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Greenford, Middlesex UB6 0HE

Principles of Clinical Measurement

Formerly *Principles of Measurement for Anaesthetists*

M.K. Sykes M.A. M.B. B.Chir. D.A. F.F.A.R.C.S. F.F.A.R.A.C.S.
M.D. Vickers M.B. B.S. M.R.C.S. L.R.C.P. D.A. F.F.A.R.C.S. F.F.A.R.A.C.S.
C.J. Hull M.B. B.S. F.F.A.R.C.S.

The first edition of this book was uniquely successful as an introductory text for anaesthetists on the basic principles of measurement techniques which could be applied to clinical practice. At that time the importance of the subject to anaesthetists had been recognized by its inclusion in the Primary FFARCS examination, but the practice of clinical measurement was mostly restricted to specialized centres. During the past ten years measurement technology has steadily improved and the use of sophisticated instruments has spread from the cardiac catheter laboratory and operating theatre to the intensive care unit, premature baby unit and obstetric unit. Furthermore, techniques which were at one time mainly the province of the cardiologist, respiratory physiologist or anaesthetist are now increasingly being used by all disciplines, whilst long-term monitoring is frequently practised on the general wards. In preparing a second edition it therefore seemed reasonable to broaden the coverage and to provide a simple text which would be of use not only to anaesthetists, but also to physicians, surgeons, paediatricians and obstetricians. Although aimed primarily at doctors, it is hoped that the text will also be of use to technicians and nurses concerned with clinical measurement or monitoring.

Contents

General principles of measurement
Units of measurement and basic mathematical concepts
Simple electronics
Biological potentials
Data processing and computation
Displaying the signal
Recorders
Electromagnetic radiation and optical measurement
The use of ultrasound in clinical measurement
Measurements using radioactive substances
Patient safety
The measurement of pressure
Direct measurement of intravascular pressures
Indirect methods for measuring arterial pressure
Measurement of gas flow and volume
Measurement of blood flow
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Descriptive and deductive statistics
Inferential statistics

Second Edition, 1980. 320 pages, 227 illustrations
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Canesten® covers all the angles
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- Free from grease, stain and smell
- A range of presentations for all body areas
- Cost-effective routine antifungal therapy

cribing information: Presentations *Cream* A white cream containing 1.0% clotrimazole. *Atomiser Spray* A clear solution containing 1.0% clotrimazole in 30% isopropanol. *Powder* A white pow
taining 1.0% clotrimazole. **Indications** All fungal skin infections due to dermatophytes, yeasts, moulds and other fungi including ringworm (tinea) infections. **Dosage and Administration** *Cre*
niser Spray Apply to the affected area 2 - 3 times daily. *Powder* as above and may also be dusted inside articles of clothing and footwear which are in contact with the infected area as a prophylac
nst re-infection. **Side-effects** Rarely, patients may briefly experience a transient local mild burning or irritation. **Precautions** Canesten atomiser spray should not be used near a
d flame, should not be allowed to come into contact with the eyes, ears or mucous membranes and should not be inhaled. **Product Licence Numbers** Cream: PL 0010/0016
niser Spray: PL 0010/0060 Powder: PL 0010/0067 **Basic NHS Prices** Cream: 20g, £1.52 50g, £3.55 Atomiser Spray: 40ml, £4.08 Powder: 30g, £1.10. Further information
ailable from Bayer UK Limited, Pharmaceutical Division, Haywards Heath, West Sussex RH16 1TP. Telephone: Haywards Heath (0444) 57911. Distributed in the Republic of Ireland by
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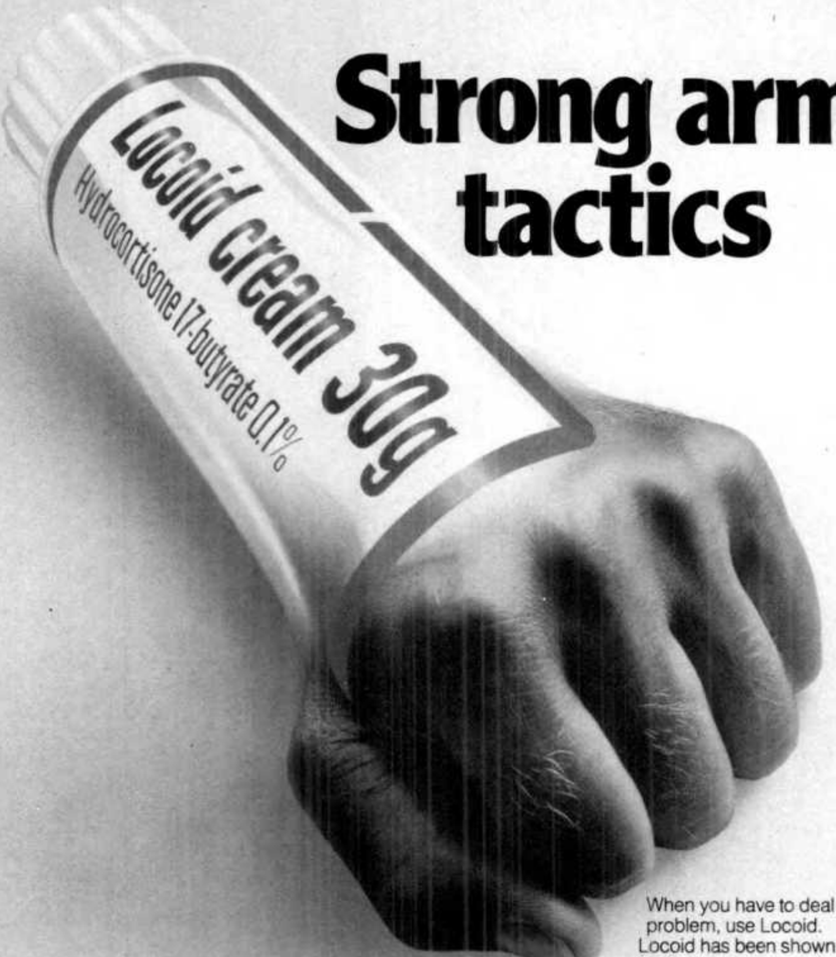
Strong arm tactics



When you have to deal with a skin problem, use Locoid.

Locoid has been shown to be more effective than triamcinolone in the treatment of eczema and psoriasis (1) and as effective and fast-acting as betamethasone 17-valerate (2, 3, 4).

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Locoid

Hydrocortisone 17-butyrate 0.1%

RELY ON ITS STRENGTH
TRUST IN ITS SAFETY

Dosage: Apply two to four times daily

Contra-Indications: In the presence of infection unless used with suitable chemotherapy. Should not be used in large amounts or for long periods in pregnancy. Avoid long-term continuous therapy in infants.
Locoid is a trademark for hydrocortisone 17-butyrate.
P/L nos.: 0166/0058; 0166/0059; 0166/0060.
30g cream/ointment £1.73. 20ml lotion £1.85.

References 1. **Dermatologica 152** (Suppl. 1) 221-229 (1976). 2. **Dermatologica 152** (Suppl. 1) 209-214 (1976). 3. **Brit. J. Clin. Pract. 27,** No. 5 (1973). 4. **I.R.C.S. Med. Sci. 4,** 533 (1976). 5. **Brit. J. Derm. 95,** 566 (1976). 6. **Brit. J. Clin. Pract. 32,** 345 (1978). 7. **Dermatologica 160,** 261-269 (1980).

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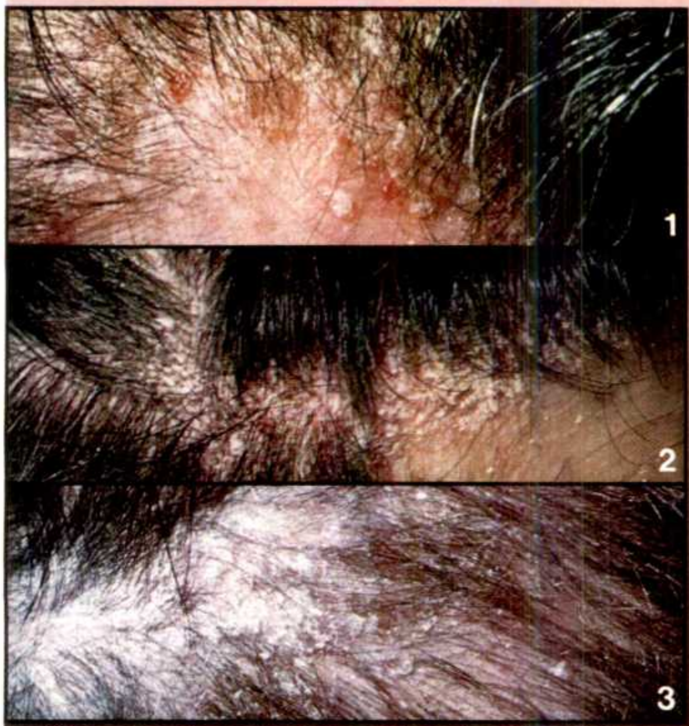
Common Disorders of the Scalp.

They all have one thing in common — the treatment



1. Psoriasis of the Scalp 2. Seborrhoeic Dermatitis of the Scalp 3. Pityriasis Capitis (Dandruff)

Polytar Liquid

Tar B.P., Cade Oil B.P.C., Coal Tar B.P.C., Arachis Oil extract of crude coal tar, Oleyl alcohol.

Acknowledged as ideal for long-term control.

NEW Polytar Plus with protein

Prescribing Information.

Presentation Polytar Liquid is a concentrated, antiseptic tar-medicated scalp cleanser containing the following active ingredients:

Tar B.P.	0.3%
Cade Oil B.P.C.	0.3%
Coal Tar B.P.C.	0.1%
Arachis Oil extract of crude coal tar	0.3%
Oleyl alcohol	1.00%

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.

Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
Polytar Liquid	65ml	57p
	150ml	82p
	350ml	£1.48
	1 litre	£3.90
Polytar Plus	150ml	90p

Product Licence Number
Polytar Liquid PL0174/5016 Polytar Plus PL0174/0037

Full information and Data sheet on request.

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Dithrocream

dithranol BP

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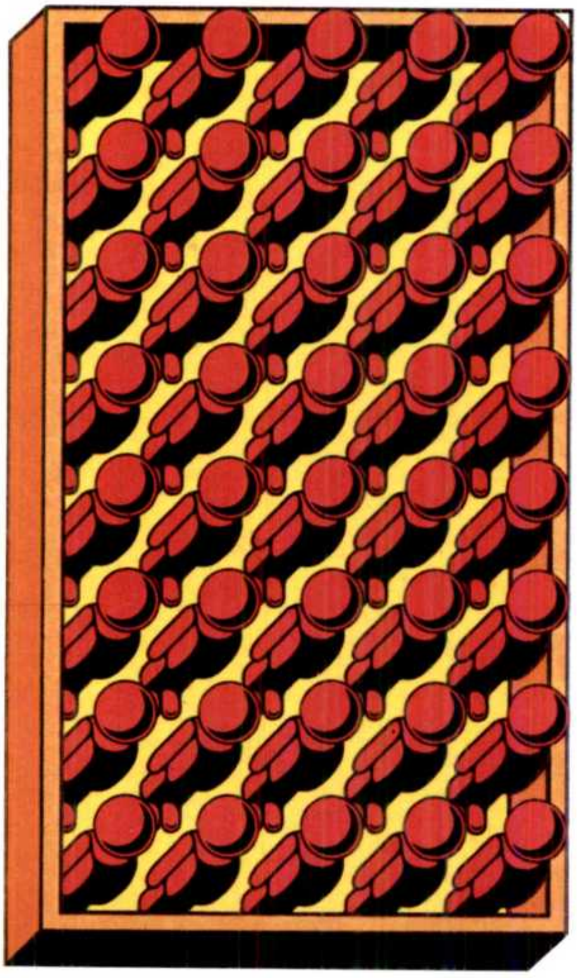
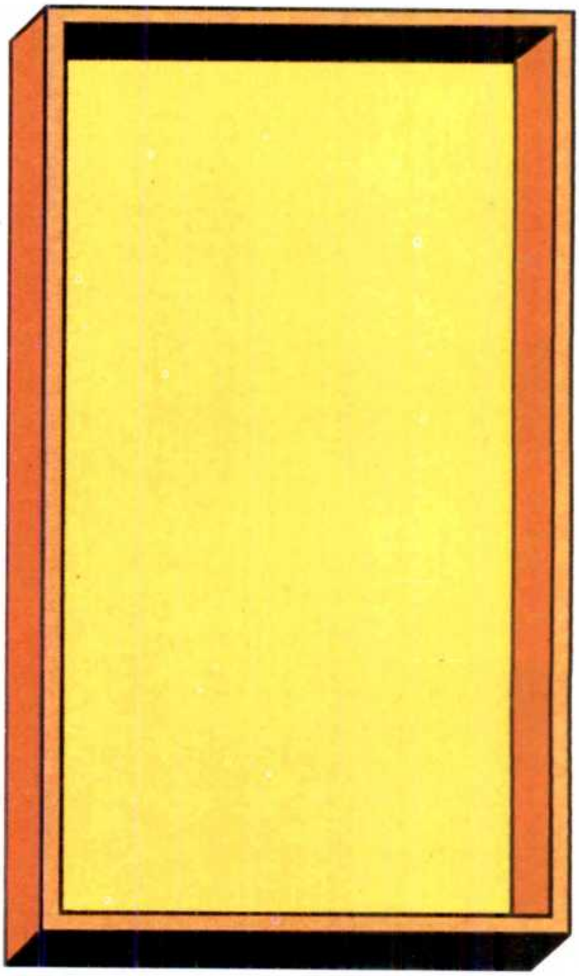


All 40 patients in the trial preferred Dithrocream



Not a single patient preferred dithranol ointment

In a recent clinical trial,[1] Dithrocream 0.25% was compared with dithranol ointment of equivalent strength in the treatment of psoriasis.

Whilst both preparations were effective, there was a unanimous patient preference for Dithrocream with all 40 patients selecting the vanishing cream formulation.

Combining effectiveness, convenience and stability, Dithrocream is the patient's choice for psoriasis treatment.

Presentation
Dithrocream 0.1%: pale yellow aqueous cream containing 0.1% Dithranol B.P.
Dithrocream 0.25%: pale yellow aqueous cream containing 0.25% Dithranol B.P.
Dithrocream Forte: pale yellow aqueous cream containing 0.5% Dithranol B.P.

Indications
For the topical treatment of subacute and chronic psoriasis including psoriasis of the scalp. Dithrocream Forte should only be used for resistant cases.

Directions for use
Apply sparingly to the psoriatic lesions preferably at night and rub in gently and carefully until absorbed. Bathe before the next application. For scalp lesions, shampoo before use. Continue treatment until the skin is smooth and clear.

Contraindications
Acute psoriasis; dithranol sensitivity.

Precautions
Keep away from the eyes. Apply to psoriatic areas only. Use cautiously after recent application of potent steroids. Wash the hands after use.

Basic N.H.S. prices:
Dithrocream 0.1% 50gm tubes £2.10
Dithrocream 0.25% 50gm tubes £2.27
Dithrocream Forte 50gm tubes £2.54

Product Licence Nos.

Dithrocream 0.1%	PL 0173/0029
Dithrocream 0.25%	PL 0173/0028
Dithrocream Forte (0.5%)	PL 0173/0027

References:

1. *British Journal of Dermatology*, 1979, **100**, 475

Full prescribing information is available from
Dermal Laboratories Limited,
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Dithrocream is a registered trade mark.

Now available in three strengths

British Journal of Dermatology (1981) **104**, 231.

Clinical and Laboratory Investigations

The kinetics of metaphase arrest in human psoriatic epidermis: an examination of optimal experimental conditions for determining the birth rate

IAN RALFS*, RODNEY DAWBER*, TERENCE RYAN*,
MARK DUFFILL† AND NICHOLAS A. WRIGHT‡

* Department of Dermatology, University of Oxford, Slade Hospital, Oxford,
† Department of Dermatology, Hamilton Hospital, New Zealand and
‡ Department of Histopathology, Royal Postgraduate Medical School, Hammersmith Hospital,
Ducane Road, London W12 0HS

Accepted for publication 16 May 1980

SUMMARY

The optimum experimental conditions have been investigated for the measurement of the birth-rate in human psoriatic epidermis using the vinca alkaloids vinblastine and vincristine. The dose response characteristics of the two drugs were assessed over a 2·5 h collection period; although the dose response characteristics differed between the two agents, a dose of 1 μg injected intradermally was considered to be optimal for both. The linearity of metaphase correction was assessed for both agents, taking biopsies for up to 10 h after injection. It was found that there was a delay period for up to an hour which must be avoided during the analysis to prevent an underestimate of the birth rate. However, linearity was then demonstrated for up to 4 h after injection. Metaphase degeneration was assessed by isolating a cohort of unlabelled cells in the G_2 phase and tracing their fate over the next 10 h. It was found that the minimum life of an arrested metaphase was of the order of 4 h, and then metaphases degenerated at the rate of approximately 0·4% per hour. It is recommended that metaphase arrest experiments in psoriatic epidermis be confined to 4 h although, if required, correction factors are available to correct for experiments which are lengthened. Finally it is shown that flux into DNA synthesis exceeds the flux into mitosis by four or five times; several explanations are considered, and it is thought that the most likely explanation involves the death of psoriatic cells in the G_2 phase.

Much of the current controversy which surrounds the measurement of proliferative rates and their interpretation in human epidermis takes origin from applying inappropriate techniques and basic misunderstanding about the assumptions which are necessary in their application. Wright (1977; 1981) has emphasized the numerous problems which beset the measurement of the cell cycle time in human epidermis using the fraction labelled mitoses (FLM) and continuous labelling methods; not

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the least of these is the ethical constraint which mediates against the taking of numerous skin biopsies after injection of tritiated thymidine (^{3}HTdR). Moreover, the use of the flash labelling index as the sole thymidine criterion of proliferative rate can be criticized because of its dependence, not only upon the rate of entry of cells into DNA synthesis, but also upon the duration of DNA synthesis itself.

For these several reasons, workers have looked to alternative methods for the assessment of proliferative status in human epidermis. Of the measurements which require only a short experimental period, there have emerged two kinetic parameters which are independent of any other measurement and will stand by themselves; these are the fluxes into DNA synthesis and into mitosis. Naturally, because for every cell that enters mitosis there is a net output of two cells, the rate of entry into mitosis is also a measurement of the birth rate or the rate of cell production; it is often proposed that the rate of entry into DNA synthesis also measures the birth rate, but cells have to pass the S and G_2 phase before reaching mitosis, and this necessitates the assumption that cells do not die, migrate, or decycle during DNA synthesis or during G_2 phase.

Duffill *et al.* (1977) have analysed the problems involved in the calculation of the rate of entry into mitosis using the stathmokinetic agent vincristine; they concluded that a major factor affecting the calculation was the age distribution of cells in the cell cycle. Appleton, Wright & Dyson (1977) proposed that the age distribution of cells in the cell cycle in layered epithelium could be predicted from the distribution of the directions of mitotic axes; in most instances where there was a single layer of proliferating cells, such as in mouse oesophageal squamous epithelium, the age distribution was of exponential type, and Duffill *et al.* (1977) and Appleton *et al.* (1977) also concluded that in tissues where multiple layers of proliferating cells were evident, such as that found in human epidermis in psoriasis, then the age distribution was also of exponential type. These considerations were applicable, not only to the measurement of the birth rate by the metaphase arrest method, but also to short term experiments involving tritiated thymidine, such as the curtailed FLM method employed by Weinstein & Frost (1968) and by Goodwin, Hamilton & Fry (1974).

It is also very important to establish the optimal experimental conditions for each metaphase arrest agent for each tissue. Wright & Appleton (1980), through a critical examination of the metaphase arrest technique, have concluded that four conditions are of paramount importance: these are (1) the choice of the optimal dose of the agent, (2) the avoidance of any delay before metaphase arrest becomes complete, (3) the demonstration of linearity of metaphase collection during the experimental period over which the birth-rate is measured and (4) the avoidance of significant degeneration of metaphases during the experimental period; if metaphases were degenerating while the birth rate was being measured, this would lead to an evident underestimate of the birth rate.

In this paper we report experimental attempts to establish the optimal conditions for metaphase arrest agents, and at the same time we report a large difference between the birth-rate as measured by the flux of cells into mitosis as indicated by the metaphase arrest method, and that evident from the flux of cells into DNA synthesis and measured by a double labelling method using tritiated thymidine.

MATERIALS AND METHODS

All patients were given full explanation concerning the procedure before informed consent was obtained. All procedures were carried out on patients with stable psoriatic plaques on the lower back and all injections were given into the periphery of the plaques. As far as was possible all injections were given between 9.00 and 11.00 a.m. in the morning. Injection, fixation and biopsy procedures were as previously described (Duffill, Wright & Shuster, 1976).

Routine paraffin embedded sections were cut at 4 μm and stained with Harris's haematoxylin. Where autoradiography was necessary, this was carried out using routine methods as previously described (Al-Dewachi *et al.*, 1975). Slides were viewed under an oil immersion lens and the measurement of proliferative indices, that is the mitotic index or the labelling index, were estimated by counting at least 3,000 germinative cells for each reading. The identification of germinative cells is subjective and the criteria used for their identification were the same as those described by Weinstein & Frost (1968) and employed by Duffill *et al.* (1976). Correction factors for the short range of β-emissions were not employed. A cell was accepted as labelled if it had at least five grains over its nucleus.

The metaphase arrest agents analysed were vincristine ('Oncovin', Lilly) and vinblastine ('Velbe', Lilly); for the measurement of labelling indices, tritiated thymidine (^{3}HTdR) of specific activity 5·0 Ci/mmol was employed.

Experiment 1: dose-response curves

To determine the optimum dose of each vinca alkaloid to use for metaphase arrest studies in psoriatic epidermis, patients with stable psoriatic plaques were given increasing doses of vincristine and vinblastine by intradermal injections into the periphery of the plaque, as described above. Doses of agents varying from 0·1 μg to 20 μg were given. At exactly 2·5 h later a biopsy was taken from each injected site using the methods described, and in each specimen the metaphase index was measured. This procedure was carried out in each of six patients for each agent. The metaphase index was then plotted against the log dose of the stathmokinetic agent.

Experimental 2: linearity studies

Fifteen patients with psoriasis were used in this study; in six of these vincristine was injected in a dose of 1 μg and biopsies were taken for up to 4 h after injection. However, in a further three patients it was possible to take biopsies for as long as 10 h after injection. In six patients vinblastine was given in a dose of 1 μg and biopsies were taken up to 4 h after injection. In each biopsy the metaphase index was determined in the usual manner and the index plotted against time after injection.

Experiment 3: the assessment of metaphase degeneration in psoriatic epidermis

This procedure was carried out in four patients; multiple sites in the periphery of stable psoriatic plaques were injected, at the same time, with 10 μCi of tritiated thymidine and 1 μg of vincristine; biopsies were taken at regular intervals for times up to 10 h after injection and processed for autoradiography. Separate counts of labelled arrested mitoses, unlabelled arrested mitoses and total arrested mitoses were made in each biopsy, and each index was plotted against time after injection of the two agents. The rationale behind the experiment is shown in Fig. 1: with the injection of tritiated thymidine, all cells in the DNA synthetic period (S phase) will become labelled; at the same time cells will become blocked in metaphase due to the effect of the vincristine. However, the cells in the G_2 phase of the cell cycle will not be labelled with tritiated thymidine and will accumulate at successive time intervals as unlabelled, arrested metaphases. After a period of time equivalent to the duration of the G_2 period, cells in the S phase at the time of injection of tritiated thymidine will begin to accumulate as arrested but labelled mitoses. This procedure enables a cohort of cells, originally in G_2, to become arrested in metaphase and be recognized by their absence of labelling. Their fate can then be monitored by successive readings of the unlabelled metaphase index with time after injection, and their degeneration characteristics assessed by this method.

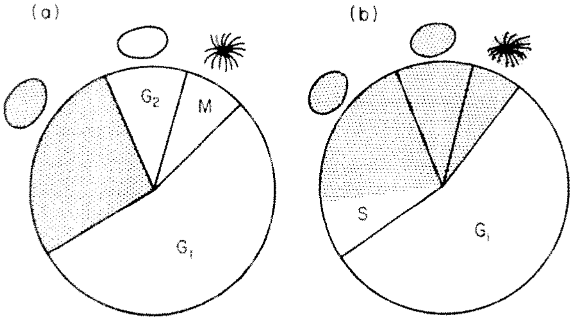

FIGURE 1. The rationale behind the experiment to measure the time of onset and rate of metaphase degeneration. (a) The kinetic situation after the initial injection of ^{3}HTdR and vincristine at time 0; all cells in the S phase are labelled and there exists a cohort of cells in G_2 which are unlabelled; these cells will appear as a cohort of arrested, but unlabelled mitoses, whose fate can then be followed. (b) The situation at 2 h after injection; labelled cells have moved through G_2 into mitosis and appear as arrested, labelled metaphases.

Experiment 4: comparison of the birth-rate as measured with the metaphase arrest method with a double-labelling technique using tritiated thymidine

The aims of this experiment were to compare the rate of entry into mitosis, as measured by the metaphase arrest method, with the rate of entry into DNA synthesis as measured by a double labelling method using tritiated thymidine.

(*a*) *Studies in different patients.*

(1) In eight patients with psoriasis, four marginal sites were injected with 1 μg of vincristine and biopsies were taken at intervals up to 2·5 h after injection. In each the metaphase index was determined and plotted against time after injection.

(2) In six different patients, two marginal sites were injected with 10 μCi of tritiated thymidine. One hour later, one site was biopsied and the other site injected with 10 μCi of tritiated thymidine. I h later this second site was biopsied and autoradiographs prepared; in each the labelling index was measured. The first injection of ^{3}HTdR will label all cells in the S phase. In the next hour, cells will flow into S out of G_1 and will be detected by the second ^{3}HTdR label. The difference between the two labelling indices will then give the rate of entry into DNA synthesis.

(*b*) *Studies on the same patient.* Four patients with psoriasis were studied.

(1) Five marginal sites on a stable psoriatic plaque were injected with 1 μg of vincristine. Biopsies were taken hourly for 5 h, sections prepared, the metaphase index measured in the usual way and plotted against time after injection of vincristine, and the birth rate calculated.

(2) In the same plaque, two marginal sites were injected with 10 μCi of tritiated thymidine. One hour later one site was biopsied while the other was injected again with tritiated thymidine. The second site was biopsied 1 h later, autoradiographs prepared and labelling indices measured in the usual way.

Analysis of metaphase arrest data

Duffill *et al.* (1977) have shown that the age distribution in psoriatic epidermis will be exponential and therefore the correct line to fit the metaphase arrest curves is:

$$\log_{10}(1+M(t)) = \log_{10} 2(t_m+t)/T_c$$

where T_c and t_m are the cell cycle time and the duration of mitosis respectively, and *M* is the metaphase index at time t.

In each case the best fit was obtained by the method of least squares.

RESULTS

(1) *Dose-response curves*

Fig. 2a shows the variation in metaphase arrest index measured at 2·5 h after injection of different doses of vincristine, and Fig. 2b shows the dose-response curve over the tested doses of vinblastine. In Fig. 2a, the metaphase index rises to a maximum at a dose of 1 μg; as the amount of vincristine was increased, the metaphase index fell. The optimum dose of vincristine, as judged by a 2·5 h collection period, is consequently 1 μg, given intradermally.

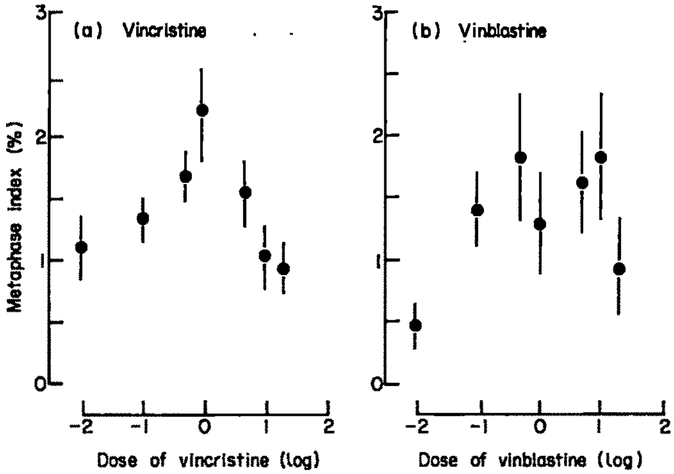

FIGURE 2. The dose-response curves for (a) vincristine and (b) vinblastine. Metaphase indices were measured at 2.5 h after injection of each agent. The readings are the mean ± s.e. mean for patients with stable plaque psoriasis.

The dose-response characteristics of vinblastine in psoriatic epidermis appear to differ somewhat from vincristine. Instead of a narrow peak in the dose-response curve, there is a broad plateau which begins at 0·1 μg, but when the dose was increased to 20 μg there appeared to be a fall in the metaphase index. A reasonable conclusion is that the optimum dose of vinblastine would be sited upon this plateau value, and would range from 0·1 μg, given intradermally (see Discussion).

(2) *Linearity studies*

Figure 3 shows some representative curves from the linearity experiments with vincristine and Fig. 4 shows a similar selection from the vinblastine series, both carried out over a period of 4 h. The dose used was 1 μg, injected intradermally. In each case there is a delay period of approximately 1 h, but thereafter collection of metaphases is linear over the 4 h experimental period. It is concluded that the early period after injection of the stathmokinetic agent, i.e. the first hour, should be excluded from the experimental period, since inclusion of these early readings would lead to an underestimate of the birth-rate. However, with each agent, metaphase collection would appear to be linear over a 4 h period.

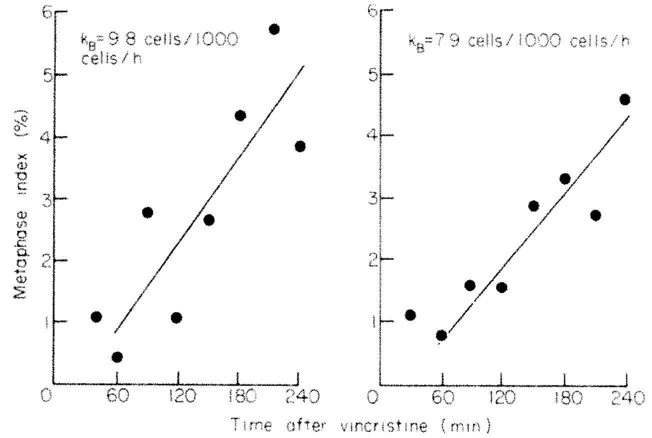

FIGURE 3. A selection of metaphase arrest curves for vincristine (1μg given itradermally) in psoriatic epidermis. The lines are fitted using equation 1.

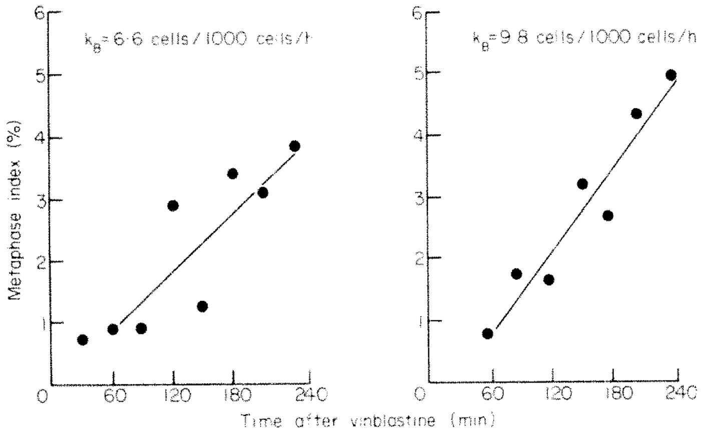

FIGURE 4. A selection of metaphase arrest curves for vinblastine (1μg given intradermally) in psoriatic epidermis; lines are fitted using equation 1.

On the other hand, if the experimental period with vincristine is prolonged to beyond 5 h, then metaphase collection is no longer linear, but the rate of accumulation slows (Fig. 5); examination of sections from specimens obtained in excess of 5 h after vincristine injection show that the metaphases are beginning to degenerate and fragment, and a reasonable interpretation for the slowing in the rate of metaphase accumulation is that the metaphases are degenerating because of the prolonged experimental period and are being lost to observation.

(3) *Measurement of the rate of metaphase degeneration*

Similar results were obtained in all four patients studied, and Fig. 6 shows results from two of these. For the first 4 h, collection of total metaphases is linear; the accumulation of unlabelled metaphases, which represents the cohort of cells originally in G_2 at the time of tritiated thymidine injection, is also linear over the first 4 h. At 100 min, which time represents the minimum duration of G_2 in psoriatic epidermis, labelled arrested metaphases begin to appear. After 4 h, however, the rate of

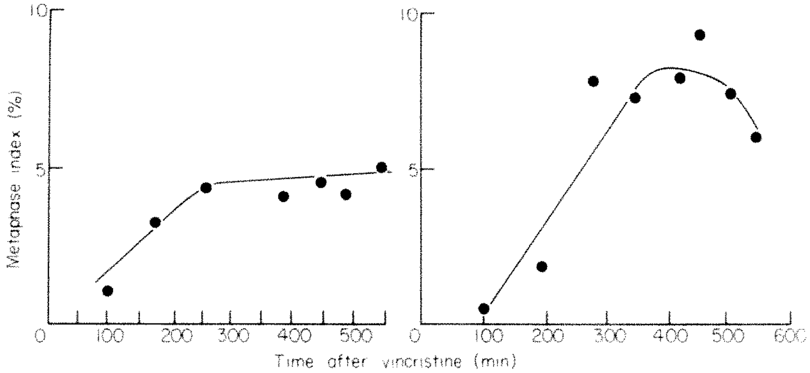

FIGURE 5. Extended metaphase arrest curves for vincristine in psoriatic epidermis.

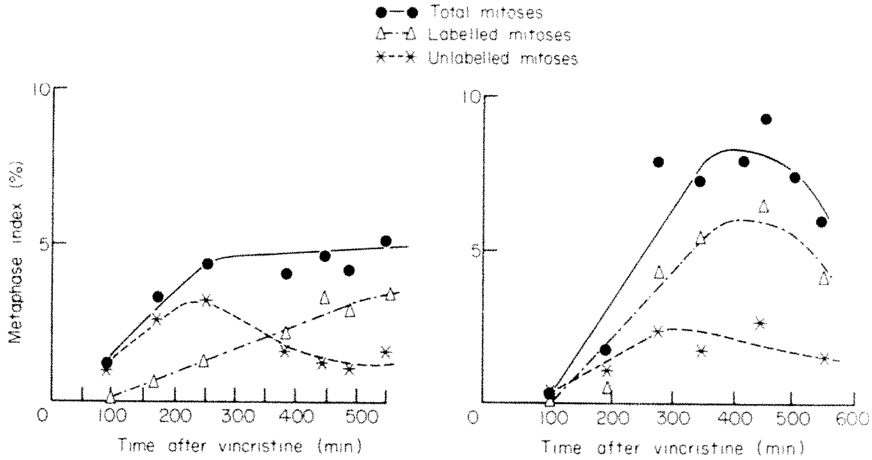

FIGURE 6. The fate of arrested labelled and unlabelled mitoses in psoriatic epidermis. After 4 h unlabelled mitoses, which represent cells originally in G_2 at the time of injection of vincristine and tritiated thymidine, begin to degenerate and to disappear. This causes a later plateau in the graph of total arrested mitoses and would lead to considerable error if all the readings were included in an estimate of the birth rate or cell cycle time.

metaphase accumulation slows; the small cohort of unlabelled metaphases, which is of course no longer being fed by new metaphases flowing into it, begins to decrease, and this reduction continues until the end of the experiment at 10 h after vincristine injection.

A reason for the reduction in the number of unlabelled metaphases can only be their degeneration and loss to observation; the rate of degeneration and loss will be approximately given by the slope of the line drawn to the data points at 4 h and after. The experimental data would indicate that vincristine arrested metaphases have a minimum life span of about 4 h and then consequently disappear at a rate of 0·4% per hour.

(4) *Comparison of the flux into mitosis with the flux into DNA synthesis*

Table 1 shows the mean values obtained for the flux into mitosis for the eight psoriatic patients studied over a period of 2·5 h. The mean value was 12·4 cells/1000 cells/h with 95% confidence

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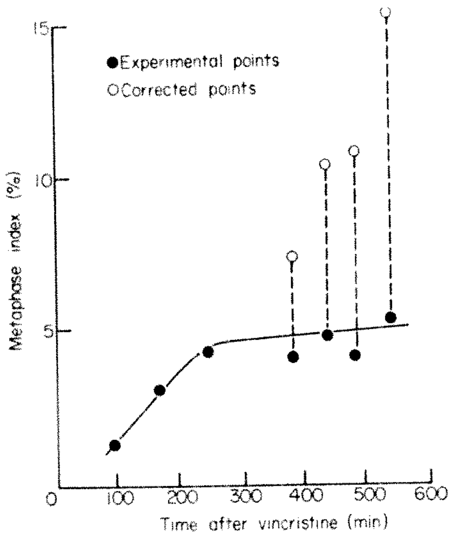

FIGURE 7. The data from patient A in Fig. 6 corrected for metaphase degeneration, using the method of Aherne & Camplejoln (1972).

interval of 7·1 and 17·8 cells/1000 cells/h. Compared with this, the mean value for the flux into DNA synthesis for the six patients studied by the double tritiated thymidine labelling method was 58 ± 11 (S.E.) cells/1000 cells/h. It is concluded that the mean flux into DNA synthesis is some five times higher than the mean flux into mitosis for these separate groups of patients.

However, psoriasis may be a very heterogenous kinetic state, with fluxes differing between individual cases. Consequently the flux into DNA synthesis and mitosis was measured in the same patients and the results are summarized in Table 2. It can be seen that the flux into mitosis varied from 7·7 cells/1000 cells/h to 9·1 cells/1000 cells/h, and in this instance the experimental period was 5 h after the injection of vincristine. The flux into DNA synthesis was again found to be much higher than the flux into mitosis, varying from 40 to 57 cells/1000 cells/h, and it can be seen that in each individual patient the mean flux into DNA synthesis is at least four times (patient 3) and as much as seven times (patient 4) higher than the flux into mitosis.

It is concluded that the flux into DNA synthesis in psoriatic epidermis considerably exceeds the flux into mitosis.

TABLE 1. The flux into DNA synthesis compared with the flux into mitosis in the epidermis of patients with psoriasis

Flux into DNA synthesis* (cells/1000 cells/h)	Flux into mitosis† (cells/1000 cells/h)
58 ± 11‡	$12{\cdot}37^{(17{\cdot}8)\S}_{(7{\cdot}1)}$

* The mean of six patients, measured with a double ^{3}HTdR labelling method.

† Measured in eight patients using the vincristine method.

‡ S.e.mean.

§ 95% Confidence intervals.

TABLE 2. The flux into DNA synthesis and into mitosis in psoriatic epidermis, measured in the same patient

Patient	Flux into DNA synthesis* (cells/1000 cells/h)	Flux into mitosis† (cells/1000 cells/h)
1	47·5	7·75 (11·9)‡ (3·8)
2	43·8	6·5 (10·7)‡ (2·5)
3	40·3	9·1 (15·2)‡ (3·4)
4	57·2	8·0 (12·4)‡ (4·3)

* Measured by a double labelling method with ^{3}HTdR.
† Measured by the metaphase arrest method using vincristine.
‡ 95% Confidence intervals for the flux into mitosis.

DISCUSSION

The strength of the stathmokinetic method lies in its ability to provide a kinetic parameter which is independent of any other consideration and consequently its use is to be preferred to that of a proliferative index, whether flash thymidine or mitotic index. These present investigations, however, have shown that, as with most other kinetic techniques, there are numerous experimental pitfalls which must be avoided. Nevertheless, Wright & Appleton (1980) have shown that, correctly employed, the stathmokinetic method gives results which compare very well with the more sophisticated, complex and time-consuming fraction of labelled mitoses method, in numerous experimental situations.

Although other workers have favoured the use of the colchicine derivative colcemid (Fisher & Wells, 1968; Fisher, 1968, Fisher & Maibach, 1972), our previous experience has indicated that the vinca alkaloids were preferable in several situations, and this is also discernable from the literature: for example Tannock (1967) showed that colcemid did not give any constant dose-response relationships and furthermore the linearity of metaphase collection in both the small intestine and in an experimental tumour was far from adequate and did not compare with that obtained with the vinca alkaloids. Consequently we have confined our studies, in the clinical situation, to the vinca alkaloids, and we have previously shown (Duffill, Wright & Shuster, 1976) that the cell cycle time as estimated by the vincristine metaphase arrest method in psoriatic epidermis also compares well with that measure by the fraction of labelled mitoses technique. Because of its intrinsic value, it becomes important to discuss the appropriate experimental conditions for the technique.

Dose-response curves

At the outset of any stathmokinetic investigation, it is important to establish the appropriate dose of the stathmokinetic agent. This should be that dose which produces a maximal metaphase arrest index and does not lead to anaphase escape or the presence of ana-telophases, in which case metaphase arrest is incomplete. This would obviously lead to an underestimate of the birth rate. It can be seen from Fig. 2 that lower doses of both vinblastine and vincristine produce low metaphase indices and in these low-dose biopsies ana-telophases are evident. However, increasing the dose leads to complete metaphase arrest, and after the peak (in the case of vincristine) or the plateau (in the case of vinblastine) there is a fall in the metaphase index, it being noted that degeneration of metaphases was commonplace in these specimens even after so short a time as 2·5 h, the possible reason for this decline in the metaphase index being an enhanced metaphase degeneration rate. It is evident from Fig. 2a that the appropriate dose of vincristine is 1 μg, but with vinblastine a generally broad plateau is evident and it would appear that 1 μg of vinblastine is also an effective and optimal dose.

Linearity studies

We have shown that vinblastine and vincristine in psoriatic epidermis give linearity of metaphase collection over a 4 h period. However, there are several important observations to be made from this study: we have shown that there is a latent period which lasts 1 h after injection of the agents during which the metaphase index did not rise. There is thus a latent period before metaphase arrest becomes effective which should be excluded from the experimental period, or else the birth rate will be underestimated if these early readings are included in the data.

Delay periods have been reported for other squamous epithelia, namely the mouse oesophagus (Duffill *et al.*, 1977). Taylor (1965) considered that the delay period after application of a metaphase arrest agent to cells in culture was itself dose-responsive. These results were considered to indicate that high doses of a stathmokinetic agent could be expected to arrest those cells already in metaphase, while lower doses were only effective upon cells entering metaphase after the application of the treatment.

The rate of metaphase degeneration

We have shown that, with vincristine in the psoriatic epidermis, the minimum life of an arrested metaphase is 4 h, after which time degeneration ensues with a decay in the rate of metaphase accumulation. This is the first such measurement to be performed in human tissues, but the results compare with previous findings in experimental animals (Aherne & Camplejohn, 1972) and in cell culture (Puck, Sanders & Petersen, 1964). Aherne & Camplejohn (1972) reported a metaphase life span of 2·6 h for mouse small intestinal crypt cells and 5·5 h for the cells of a hamster lymphoma, both with vincristine. Puck *et al.* (1964) found a life span of 6 h for CHO cells in culture with colchicine.

There is no doubt that Fisher (Fisher, 1968; Fisher & Wells, 1968; Fisher & Maibach, 1972), who used arrest periods up to 12 h, incurred such errors in using colcemid in cream form.

However, Aherne & Camplejohn (1972) proposed a correction procedure to account for such degeneration; assuming that metaphase degeneration is a random process, the arrested metaphase index at any time after application of the agent can be corrected by multiplying it by:

$$\exp\,[\lambda-\beta(\mathrm{t}-\mathrm{m})]$$

where m is the minimum life span of an arested metaphase, t is the time after injection and β and λ are the rate at which metaphases accumulate (i.e. the birth rate) and degenerate respectively. Figure 7 shows the metaphase arrest line corrected by this procedure.

Fisher (Fisher 1968; Fisher & Wells, 1968) reported a value for the birth rate in normal human epidermis of 0·027 cells/1000 cells/h. He used a single reading at different times after application of colcemid cream; this value has been quoted many times now (see, for example, Wright, 1981), but, if the criteria we have reported also applied in normal epidermis, then this value should be changed to 0·17 cells/1000 cells/h, a considerable difference.

Inequality of fluxes into DNA synthesis and mitosis

Goodwin, Hamilton & Fry (1973) reported some very interesting and rather incongruous data comparing labelling and mitotic indices in normal, uninvolved and involved psoriatic epidermis. They found that the ratio of labelled to mitotic psoriatic germinative cells was of the order of 27 : 1. Our measurements of the duration of mitosis in psoriatic epidermis reflect values of about 1–1·5 h, and of the duration of DNA synthesis, about 7·5 h. Thus, on the basis of the duration of the respective phases alone, the ratio should be 6 or 7: 1.

We have shown that the rate of entry into DNA synthesis is 4 or 5 times higher than the rate of entry into mitosis in psoriatic epidermis; this would agree with the ratio of 27: 1 by Goodwin *et al.* (1973). Although the data are internally consistent, they offer no suggestion as to the likely mechanism of the effect. Moreover, the incongruities between the labelling and mitotic indices in uninvolved and normal epidermis were 290:1 and 480:1 respectively. If our findings in psoriatic epidermis are reflected in uninvolved and normal epidermis, then this would indicate very wide discrepancies in the rates of entry into DNA synthesis and mitosis.

We can at this stage only speculate on the mechanism of this observation. There are three possible mechanisms, and the first of these may be that the observation can be explained by the exponential age distribution which applies in psoriasis (Duffill *et al.*, 1977; Appleton *et al.*, 1977). Although the rate of entry into DNA synthesis will be larger than the rate of entry into mitosis, based upon these considerations, this difference should be only approximately 1·4 times. Secondly, there is the possibility that the first injection of tritiated thymidine is stimulating the flux of cells into DNA synthesis which will be picked up by the second injection of the double labelling procedure. There is some previous evidence for this in that Moller & Larsen (1974) reported that an injection of tritiated thymidine stimulated cells into DNA synthesis some 4–5 h after injection in hamster cheek-pouch squamous epithelium. However, the time course of this present response does not appear to fit with Moller & Larsen's observation, and this explanation does not explain the 27: 1 ratio of labelling to mitotic indices reported by Goodwin *et al.* (1973). Thirdly, we should consider that the discrepancy reflects a loss of cells in the G_2 period. It is considered unlikely that the observations reflect a blockage of cells in the G_2 period as suggested by Gelfant (1976), because such a situation would lead to the renewal system rapidly running down. Furthermore it is considered unlikely that the loss could be caused by migration of cells in G_2 into the non-proliferating supragerminative layers, since some 75% of thymidine labelled cells would need to migrate in a period of about 9 h. The alternative explanation is that there may be death of such cells in the G_2 phase, which would certainly account for the observations. Claesson & Hartmann (1976) have reported that 17–19% of thymus cells died between the S phase and mitosis, and it may be that a similar kinetic situation obtains in psoriatic epidermis. Further work is needed on this topic.

Conclusions

It is concluded that the metaphase arrest method can be a reliable estimator of the birth rate in psoriatic epidermis provided several experimental and theoretical points are remembered. These include the use of an optimum dose of the stathmokinetic agent, the avoidance of a delay period, proof of linearity and avoidance of metaphase degeneration. We also maintain from earlier work (Duffill *et al.*, 1977), that the use of the correct age distribution is important when fitting the line to the data. The drawbacks of the method in human skin include the unfortunate observation (Camplejohn & Gelfant, 1981) that the technique does not give satisfactory results in normal epidermis, and also that it needs a series of biopsies after injection for a single measurement of the birth rate; consequently there will be difficulty in following changes in proliferative rates in the same patient after some induced change, such as treatment.

It has also been shown that double labelling techniques give values for the flux into DNA synthesis which are much higher than the flux into mitosis. Providing this is not due to some artefact produced by the injection of tritiated thymidine, the results may indicate that significant death of cells is occurring in psoriatic epidermis.

ACKNOWLEDGMENT

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An attempt to use vincristine and colcemid to measure proliferative rates in normal human epidermis *in vivo*

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SUMMARY

A range of doses of vincristine (0·5–10 μg) or colcemid (5–100 μg) injected intradermally into normal volunteers failed to yield any significant accumulation of arrested mitoses. This was despite an apparently successful block of cells in metaphase by the highest three doses of each drug. Possible reasons for the failure to see accumulation are discussed. The results suggest that normal human epidermis is not a suitable system for application of the metaphase-arrest technique using intradermal injection.

The technique of metaphase-arrest with agents such as colcemid or the vinca alkaloids, offers the possibility of obtaining a variety of useful information about the kinetics of normal human epidermis. For example, this method enables the rate of cell production and the duration of mitosis to be estimated. In addition the technique, in combination with labelling studies, allows the flux of cells into S and mitosis to be compared in a manner similar to that described by Ralfs *et al.* (1981) for psoriatic epidermis. In these studies Ralfs *et al.* (1980) have demonstrated a five-fold higher rate of entry of cells into S than into mitosis. An earlier study of Goodwin, Hamilton & Fry (1973) had shown that the ratio of labelled cells: mitotic cells was 27:1 for psoriatic and 450:1 for normal epidermis. Large labelled cell : mitotic cell ratios have more recently been confirmed in normal epidermis by the more detailed studies of Camplejohn & Gelfant (1981). Thus, because of this vast excess of labelled compared with mitotic cells in normal epidermis, one would suspect that an even greater imbalance may exist in the fluxes of cells into S and mitosis. If this is so, the validity of many studies in which data from either simple labelling or double labelling experiments were used in the calculation of kinetic parameters such as cell production rates, potential doubling times or growth fraction (Heenen, Achten & Galand, 1973; Steigleder, Schumann & Lennartz, 1973), is in doubt.

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The metaphase arrest technique has already been applied successfully to psoriatic epidermis (Duffill *et al.*, 1977). However, as pointed out by Wright & Appleton (1980), care must be taken to find the optimal experimental conditions for such a study. Due to the long period of metaphase accumulation employed in earlier studies on normal human epidermis by Fisher (1968), it is probable that cell production rate was seriously underestimated due to degeneration of arrested metaphases (Aherne & Camplejohn 1972; Ralfs *et al.*, 1981). Also, the techniques employed by Fisher (1968) of applying colcemid in a cream to the skin surface would seem to involve considerable restrictions in the ability to deliver a controlled dose of agent at a precise time to the proliferative cells of the epidermis. For this reason, a carefully controlled method of intradermal injections was used in the present study, which was the first stage in an attempt to find the optimal conditions for metaphase arrest in normal human epidermis.

MATERIALS AND METHOD

Five male volunteers with an average age of 25 years (range 22–31) were injected at five sites on the inner forearm with four doses of vincristine (0·5, 1·0, 5 and 10 μg) and in one site with saline. Five other volunteers were injected with four doses of colcemid (5, 10, 50 and 100 μg) and in one area with saline. All injections were performed between 8 and 9 a.m. Injections were carried out by inserting a half-inch 30 gauge needle superficially under the epidermis and advancing it so that the area of the bleb produced by the injection was removed from the point of entry of the needle. In each case a volume of 0·1 ml was injected slowly with the bevel of the needle facing upwards. The sites of injection were at least 3·5 cm apart and each site was ringed at the time of injection. Biopsy procedures were carried out 3 h after injection with a 4 mm punch with 1% lignocaine as local anaesthetic. The specimens were fixed overnight in Bouin's and embedded for paraffin sectioning. Sections were cut at 5 μm and stained with haematoxylin and eosin. The number of mitoses and pyknotic cells were counted in each biopsy by scanning 200 mm of epidermis. The results were expressed in terms of mitotic activity (mitoses/100 mm epidermis).

TABLE 1. Mitotic counts following injection of a range of vincristine doses

	Dose of vincristine (μg)				
Volunteer	0	0·5	1·0	5	10
RK	1·0	14·0	12·8	13·0	13·3
	(2/201·4)	(28/200·1)	(26/203·6)	(26/200·0)	(30/226·3)
MG	17·3	16·3	10·8	1·8	12·3
	(38/219·3)	(36/221·5)	(23/213·8)	(4/219·8)	(25/203·8)
DN	30·2	3·0	4·5	9·4	2·4
	(65/215·5)	(6/202·6)	(10/223·5)	(20/212·3)	(5/211·1)
MA	2·0	22·4	20·2	23·9	10·8
	(4/202·2)	(45/201·3)	(42/207·6)	(54/226·1)	(24/221·5)
DY	6·5	30·7	39·3	29·3	24·7
	(13/200·7)	(65/211·6)	(82/208·4)	(61/208·4)	(54/218·2)
Mean±s.e.	11·4±5·3	17·1±4·8	17·5±4·8	15·5±5·0	12·7±4·6
	(122/1039)	(178/1037)	(183/1057)	(165/1067)	(138/1081)

Results are given as mitotic activity (mitoses/100 mm epidermis) with the actual counts in brackets (mitoses/mm of epidermis counted).

RESULTS

Tables 1 and 2 give the counts of mitoses per 100 mm of epidermis for the various doses of vincristine and colcemid. No significant accumulation of metaphases was seen with any dose of either drug. (Comparing each drug with its relevant control using paired t-tests P was always greater than 0·5.) This is despite the fact that an apparently effective block of cells in metaphase was achieved, as demonstrated by the complete absence of ana- and telophases with all but the lowest doses of both drugs (see Table 3).

In view of the possibility that failure to see accumulation was due to a rapid degeneration of arrested metaphases, a count of pyknotic cells in each sample was carried out (Table 4). An increase of pyknotic cells was noted with vincristine and with three of the doses this difference was significant as indicated in Table 4.

TABLE 2. Mitotic counts following injection of a range of colcemid doses

	Dose of colcemid (μg)				
Volunteer	0	5	10	50	100
TP	19·9 (41/205·7)	6·4 (13/204·0)	6·4 (13/204·3)	6·7 (15/233·8)	3·3 (7/213·6)
KB	7·0 (15/215·7)	13·4 (28/209·2)	20·4 (42/205·7)	35·6 (72/202·3)	13·7 (28/204·4)
BJ	13·0 (26/200·1)	10·0 (22/220·3)	12·7 (26/205·4)	1·4 (3/213·4)	3·0 (6/202·7)
CB	30·7 (69/224·5)	38·6 (78/202·3)	25·0 (57/228·1)	26·2 (55/210·1)	24·4 (49/200·9)
MM	12.3 (26/211·8)	9·3 (19/204·8)	10·4 (21/201·2)	8·2 (18/218·4)	9·7 (20/205·4)
Mean ± s.e.	16·6 ± 4·1 (177/1058)	15·5 ± 5·9 (160/1041)	15·0 ± 3·4 (159/1045)	15·6 ± 6·5 (163/1078)	10·8 ± 3·9 (110/1027)

Results are given as mitotic activity (mitoses/100 mm epidermis) with the actual counts in brackets (mitoses/mm of epidermis counted).

TABLE 3. Ana- and telophase counts

Vincristine dose (μg)	0	0·5	1·0	5	10
Number ana- and telophases / Total number mitoses	25/122	1/170	0/183	0/165	0/138

Colcemid dose (μg)	0	5	10	50	100
Number ana-and telophases / Total number mitoses	21/177	1/160	0/159	0/163	0/110

The results are given as the combined count of ana- and telophases and total mitoses for each group of five observations.

TABLE 4. Pyknotic counts as pyknoses/100 mm epidermis: mean ± s.e. for each group observations

Vincristine dose (μg)	0	0·5	1·0	5	10
Pyknotic cells/100 mm	0·8 ± 1·0	3·6 ± 1·0*	4·3 ± 1·7	6·2 ± 1·8*	4·2 ± 0·8**
Colcemid dose (μg)	0	5	10	50	100
Pyknotic cells/100 mm	2·1 ± 0·6	3·2 ± 0·7	2·9 ± 0·6	3·9 ± 0·8	3·4 ± 0·7

Values significantly different from the control group—*at the $P < 0·05$ and ** at the $P < 0·01$ level—using paired t-tests.

DISCUSSION

It is difficult to explain the failure to obtain accumulation of metaphases in normal human epidermis after injection of vincristine or colcemid. The variability between individuals within each group was considerable, and as the rate of cell production in normal human epidermis is low, one could suggest that the variability between individual volunteers had masked any accumulation which took place. However, if this were the only reason, one would perhaps have expected some tendency for the drug-treated samples to be higher and this was not the case. It seems unlikely that many cells were escaping the block since no ana- or telophases were seen with any of the three highest doses of each drug. Also, doses of vincristine within the range used here and delivered in a similar manner, achieved successful accumulation of metaphases in psoriatic epidermis (Duffill *et al.*, 1977; Ralfs *et al.*, 1981). The counts of pyknotic nuclei show small increases compared to the controls, which raises the possibility that a very rapid onset of metaphase degeneration may have played a role in the failure to achieve significant metaphase accumulation. However it should be stressed that the numbers of pyknotic cells seen were small and these counts do not show conclusively whether degeneration was indeed a factor in the failure to see accumulation. Further, Ralfs *et al.* (1980) did not see any degeneration of arrested metaphases with a dose of 1 μg vincristine in psoriatic epidermis until 5 h after injection. The study of Ralfs *et al.* (1980) did, however, demonstrate a long lag-period of 1 h after injection of vincristine before accumulation began in psoriatic epidermis. Such a situation in normal human epidermis might contribute to the failure to see significant accumulation of cells in metaphase.

In summary, no single reason for this failure is apparent but factors such as low rate of cell production, high variability and a long lag-phase may make normal human epidermis unsuitable for application of the metaphase-arrest technique. Any attempt to lengthen the period of arrest significantly would most probably lead to serious problems with metaphase degeneration.

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Pityriasis rubra pilaris: epidermal cell kinetics

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SUMMARY

Epidermal proliferative kinetics were studied in a patient with pityriasis rubra pilaris. Elevated proliferative indices (flash labelling and mitotic indices) were apparent compared with normal epidermis and measurements of the birth rate indicate that the rate of epidermal cell production was similar to that found in psoriasis, and equalled 11·8 cells/1000 germinative cells/h; this suggests a cell cycle time of 60 h.

Contemporaneous measurements of the rate of entry into DNA synthesis showed values similar to the rate of entry into mitosis; hence there is no G_2 cell loss as appears to occur in the hyperproliferative psoriatic epidermis.

For some time there has been evidence that the epidermis in pityriasis rubra pilaris (P.R.P.) is hyperproliferative. Porter & Shuster (1968) injected ^{14}C-labelled methionine and glycine intradermally; following this the peak loss of these labelled amino acids from the surface was, at 7 days, very similar to that found in psoriatic plaques. Several investigators have shown elevation in the flash labelling index (I_s) following intradermal tritiated thymidine (^{3}HTdR); Niemi *et al.* (1976) studied thirty-one patients with P.R.P. and found a mean of 8·9%, compared with normal levels of around 3%; Marks & Griffiths (1973) reported a higher value of 27·3%. At this time current thinking suggested that cell proliferation in P.R.P. was enhanced, being somewhere between normal and that evident in psoriasis, thus explaining the evident differences in clinical appearance and response to therapy.

We recently had the opportunity to study the cell kinetics of one patient with typical P.R.P. However, it was possible not only to measure the mitotic and labelling indices but also to examine the rate of entry into mitosis using the metaphase arrest method with vincristine, and the rate of entry into DNA synthesis using a double labelling technique with two injections of ^{3}HTdR. These two latter measurements are much more informative of the actual proliferative rate than the mere proliferative indices in that they are hard kinetic parameters and stand by themselves, not being dependent on any other parameter.

CASE HISTORY

A 79-year-old white female was well, without any preexisting skin disease, until 2 years previously. At that time she suddenly developed areas of scaly erythema on the chest; these rapidly enlarged and

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within 4 weeks she was erythrodermic, although islands of normal skin remained for a few weeks before her integument was completely involved. At the time of presentation to the dermatology clinic, in addition to the erythroderma, she showed plantar-palmar hyperkeratosis with yellow discolouration, ectropion, and gross terminal subungual hyperkeratosis. The clinical diagnosis of P.R.P. was supported by biopsy which showed patchy parakeratosis, acanthosis, and dermal infiltration of lymphocytes and histiocytes.

At the time of cell kinetic studies the clinical condition was stable.

METHODS

(1) *Mitotic index* (I_m)

In lesional epidermis a 4 mm punch biopsy was taken under 2% lignocaine anaesthesia. This was fixed in Carnoy's solution, processed in the standard manner and stained with haematoxylin and eosin. Mitoses were enumerated in counts of at least 3000 germinative cells, made under oil immersion. The criteria of Weinstein & Frost (1968) were used to identify germinative cells. The I_m is then equal to:

$$\frac{\text{Number of mitoses}}{\text{Number of germinative cells}} \times 100$$

(2) *Flash labelling index* (I_s)

Tritiated thymidine (^{3}HTdR) 10 μCi was injected intradermally into lesional epidermis. This site was biopsied 1 h later and autoradiographs were prepared in the usual manner using Kodak AR10 stripping film (Al-Dewachi *et al.* 1975); counts were made under oil immersion of the number of labelled cells in at least 3000 germinative cells. A cell was regarded as labelled if it had five or more grains localized over its nucleus. The I_s is then:

$$\frac{\text{Number of labelled cells}}{\text{Number of germinative cells}} \times 100$$

(3) *Rate of entry into mitosis*

Five sites in lesional epidermis were injected intradermally with 1 μg vincristine sulphate, following the method of Duffill, Wright & Shuster (1976). These sites were then biopsied at hourly intervals and processed as for the mitotic index. Counts were made of the number of arrested metaphases in at least 3000 germinative cells. A line was then fitted to the data using the least squares method. The slope of the line then gives the rate of accumulation of metaphases, which is equivalent to the rate of entry into mitosis.

(4) *Rate of entry into DNA synthesis*

Two sites in lesional epidermis were injected with 10 μCi ^{3}HTdR; at 1 h later one site was biopsied whilst the other received a further injection of 10 μCi ^{3}HTdR. This second site was biopsied after a further hour. The biopsies were processed and counted as for the I_s measurements. The difference between the 1 h and 2 h labelling indices will reflect the number of cells which have entered DNA synthesis during the interval between the biopsies, i.e. the rate of entry per hour.

RESULTS

(1) Mitotic index: 1%.

(2) Flash labelling index: 14·5%.

(3) Entry into mitosis: 11·5 cells/1000 germinative cells/h.

(4) Entry into DNA synthesis: 13 cells/1000 germinative cells/h.

DISCUSSION

The cell kinetic parameters of one patient with P.R.P. support the concept of a hyperproliferative state.

Little has been published on the mitotic index of normal epidermis; a figure of 0·156% (Pinkus, 1952) is not directly comparable to this study as this was a percentage of all epidermal cells and not a percentage of germinative cells.

Furthermore, the identification of a germinative cell is largely subjective and has given rise to some controversy (Weinstein, 1975). The same problem of the germinative cell definition applies to the labelling index. The normal figures quoted for this vary from 3·5% (Weinstein & McCullough, 1973) to 5% (Epstein & Maibach, 1965); in psoriasis reported I_s values vary from 11% (Goodwin, Hamilton & Fry, 1974) to 23% (Weinstein, 1968); in a previous study of P.R.P., I_s was between 7·3 and 11·1% with a mean of 8·9% (Niemi *et al.*, 1976). This present patient showed a somewhat higher I_s at 14·5%. Both the I_s and I_m values would indicate that this patient's epidermis is hyperproliferative.

The ratio of mitoses to flash labelled cells bypasses the problem of the identification of labelled cells (since the same population is included in the counts by the same observer). This ratio has been calculated for psoriasis (Goodwin, Hamilton & Fry, 1973); in plaque epidermis the ratio was 1:25. This discrepancy cannot be wholly explained on differences in the duration of mitosis and of DNA synthesis alone (Ralfs *et al.*, 1981), and it is considered that, in psoriasis, many cells are lost in the G_2 phase, never entering mitosis. In this patient the ratio of mitotic to labelled cells is 1:14·5, and this ratio could most reasonably be explained by the differences in the duration of mitosis and of DNA synthesis.

The rate parameters support this proposed difference from psoriasis. The birth rate of cells, measured by the vincristine metaphase arrest method, was 11·5 cells/1000 germinative cells/h, very similar to the level found in psoriasis of 12·5 cells/1000 germinative cells/h (Duffill *et al.*, 1976). However, the rate of entry into DNA synthesis was very different; in six patients with psoriasis it was 58 cells/1000 cells/h (Ralfs *et al.*, 1981), whereas in P.R.P. the value was 13 cells/1000 cells/h. This suggests that there is no loss of cells in G_2, but that all cells that go into DNA synthesis later enter mitosis. On the basis of the exponential age distribution, which is considered to be appropriate for epidermis containing several layers of proliferating cells (Duffill *et al.*, 1977), the rate of entry into DNA synthesis should be slightly higher than the rate of entry into mitosis, and indeed this is so (see Results). This difference in cell kinetics may be related to the variable response to antimitotic therapy in P.R.P. as compared to psoriasis.

A birth rate (K_B) of 11·5 cells/1000 cells/h is equivalent to a rate of 0·0115 cells/cell/h, and will give, from the equation:

$$T_c = \ln(1 + I_p)K_B$$

(where T_c is the cell cycle time and I_p the growth fraction) a cell cycle time of 60 h, compared with a value of 54 h in psoriasis (Duffill *et al.*, 1976). In this calculation the growth fraction is assumed to be unity, and the age distribution is exponential, both of which assumptions seem reasonable for psoriasis (Duffill *et al.*, 1976, 1977).

This patient with P.R.P. shows proliferative indices similar to those observed in psoriasis, and moreover a direct estimation of the birth rate, which has not been previously reported, shows a value very similar to that found in psoriasis. However, there is no apparent discrepancy between rates of entry into DNA synthesis and mitosis, and conceivably P.R.P. epidermis is not subject to the cell deletion which may occur in psoriatic epidermis.

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Pityriasis rubra pilaris and retinol-binding protein

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SUMMARY

Serum levels of retinol-binding protein (the specific carrier of vitamin A) were measured in eleven patients with pityriasis rubra pilaris and in some of their close relatives. The level of retinol-binding protein was markedly reduced in every patient, and in some of the relatives. It is postulated that defective synthesis of retinol-binding protein is a biochemical marker for pityriasis rubra pilaris, probably transmitted as a Mendelian dominant.

Pityriasis rubra pilaris (PRP) is a rare dermatosis characterized by abnormalities of the horny layer of the epidermis, due mainly to an accelerated turnover of follicular keratinocytes.

Genetic transmission of the disease as a Mendelian dominant has not yet been proved, but affected subjects often belong to consecutive generations; on the other hand many patients have no family history of the disease. The biochemical defect in PRP has not yet been identified. Some authors (Frazier & Hu, 1931) have emphasized the importance of vitamin A deficiency in the pathogenesis of PRP but a relationship between PRP and vitamin A deficiency remains unproven (Leitner & Ford, 1947). It is known for instance that the clinical picture of vitamin A deficiency is not the same as that of PRP (Lowethal, 1933) and volunteers maintained on a vitamin A deficient diet for 2 years showed no symptoms of PRP (Sullivan & Evans, 1945; Hume & Krebs, 1949). In PRP patients the serum level of vitamin A is often normal, and last but not least, the therapeutic administration of high doses of vitamin A is almost always ineffective.

It is known (Peterson, 1971; Goodman, 1976) that vitamin A circulates in human plasma as retinol (vitamin A alcohol), bound to a specific carrier protein, retinol-binding protein (RBP). Purified RBP has an alpha-1 mobility on electrophoresis and a molecular weight of approximately 21,000–22,000. RBP circulates in plasma as a protein-retinol-protein complex with prealbumin of an apparent molecular weight of 60,000–70,000; 96% of the RBP is in the complex form, bound to retinol and prealbumin (holo-RBP).

The aim of our study was to evaluate the possible pathogenetic role of the specific vitamin A transport protein in subjects with pityriasis rubra pilaris.

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METHODS

Serum levels of RBP were determined in eleven subjects (seven females and four males) with PRP. Their ages ranged from 4 to 63 years. In four cases RBP were measured in other members of the family. In every patient the diagnosis of PRP was confirmed histologically. None had hepatic or renal disease. In three patients serum levels of RBP were also checked after oral administration of vitamin A (200,000 i.u. daily for 3 consecutive days).

The control group consisted of fifty healthy individuals of both sexes (aged 18–35 years). Venous blood samples were collected and the sera were stored at −30°C until examined. RBP serum concentration was determined by single radial immunodiffusion on cellulose acetate strips (Agostoni, Stabilini and Vergani, 1970). Since the mean RBP concentrations differed in normal males and females, the RBP levels in PRP patients are reported as a percentage of the normal mean value for the corresponding sex.

RESULTS

Healthy subjects

The mean serum RBP value in the control group was 7·02 ± s.d. 1·04 mg/100 ml in males and 5·06 ± s.d. 0·5 mg/100 ml in females ($P<0\cdot 001$); the serum concentration expressed as a percentage of the normal mean value of the corresponding sex was 100. 1 + s.d. 12·07%.

PRP patients

The results obtained in the PRP patients are summarized in Table 1, and RBP levels in relatives of some of the patients studied are shown in Table 2.

In every PRP patient RBP was markedly descreased. The mean level (58·7% ± s.d. 16·2) was significantly lower than in the control group ($P<0\cdot 001$).

In cases 3, 5 and 7 a low level of serum RBP was demonstrated in other members of the family who were clinically free of any dermatological sign of PRP. The mean value of RBP in the relatives examined was significantly decreased (78·5% ± s.d. 23·1%) compared to normal controls ($P<0.05$).

TABLE 1. Retinol-binding protein levels in eleven patients with pityriasis rubra pilaris, expressed as a percentage of values in healthy subjects of the same sex

Patient	Age	Sex	RBP%	RBP% after vit. A administration
1	63	M	69	
2	27	F	43	
3	15	F	62·5	
4	4	M	38	
5	18	F	39	40
6	29	F		
7	13	M	83	83
8	15	F	34	40
9	58	F	68	
10	16	F	73·5	
11	4	M	71·5	

TABLE 2. Retinol binding protein levels in relatives of patients with pityriasis rubra pilaris, expressed as a percentage of values in healthy subjects of the same sex

	RBP%			
Patient	Father	Mother	Brothers	Sisters
3	—	73	57, 42	—
5	—	45	—	—
7	128	96	—	70, 86, 81 70, 84
8	—	90	—	98

In three cases (5, 7 and 8) the RBP levels remained unchanged after oral administration of high doses of vitamin A. Since RBP not bound to retinol (apo-RBP) is quickly excreted by the kidney, this suggests that the RBP deficiency in PRP is not due to a deficient retinol supply.

DISCUSSION

Every patient with PRP had a serum level of RBP which was decreased, and this suggests that the supply of retinol to the epidermis was probably decreased in these patients. The low level of RBP could explain the lack of clinical effect of oral or parenteral vitamin A administration in PRP patients, while good results have been reported from topical applications of vitamin A lotion under occlusive dressing (Lamar & Gaethe, 1964) and better results still from topical treatment with retinoic acid (active form of retinol) (Günther, 1972).

Recently we and others have treated our PRP patients orally with the aromatic derivative of retinoic acid (Tigason, RO-10-9359) which produced evident clinical improvement, probably by bypassing the serum transport of vitamin A bound to RBP.

Some authors (Leitner & Ford, 1947; Kierland & Kulwin, 1950; Davidson, Winkelmann & Kierland, 1969) have suggested that PRP is transmitted as a Mendelian dominant. In this paper, which reports a significantly low level of RBP in relatives of PRP patients, we postulate that a defective synthesis of RBP is the biochemical marker of the genetic defect. The mechanism which triggers the clinical onset of the disease in only some of the subjects with low RBP levels is still unknown.

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The relevance of morphometry in the differential diagnosis of cutaneous T cell lymphomas

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SUMMARY

Morphometric analysis of lymphoid cells in the skin was used to differentiate between cutaneous T cell lymphomas (CTCL), i.e. mycosis fungoides (MF) and Sézary syndrome (SS), and chronic benign skin diseases. In electronmicrographs of the skin lesions from twenty patients with CTCL (group I), fourteen patients with chronic benign skin diseases (group II) and twenty-nine patients suspected of CTCL (group III), the degree of nuclear indentation of lymphoid cells, expressed as the nuclear contour index (NCI), was measured.

Analysis of the NCI histograms of the infiltrating cells of group I and group II permitted us to derive classification criteria for allocating all patients correctly with a high probability ($\geq 95\%$) in the appropriate group. Only one case was classified with a low probability (71%). The classification criteria are based on the presence of cerebriform mononuclear cells (CMC) with highly indented nuclei ($NCI \geqslant 11{\cdot}5$) and the frequency distribution of CMC in the skin infiltrates expressed as the 25th and the 70th percentiles of the NCI histograms (P25 + P70).

When these criteria were tested on twenty-nine patients suspected of CTCL, twenty cases were classified as malignant, nine as benign. During the follow-up period, out of the twenty patients classified as malignant, seventeen patients appeared to have or develop MF, whereas two patients had lymphomatoid papulosis and one patient is still suspected of MF. Of the nine patients classified as benign, eight patients were proven to have benign skin diseases whereas one developed MF in the follow-up period of up to 4 years.

The classification results based on morphometry proved to be more sensitive than those based on DNA cytophotometry. It is concluded that morphometric analysis of lymphoid cells in the skin is of diagnostic relevance in the differential diagnosis of CTCL.

Mycosis fungoides (MF) and Sézary syndrome (SS) are cutaneous T cell lymphomas (CTCL) characterized by the presence of T lymphocytes with highly indented nuclei, the cerebriform mononuclear cells (CMC), in the skin.

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Histologically the early diagnosis of MF, i.e. the premycotic and early plaque stage, may be difficult since the histological picture often resembles chronic non-specific dermatitis (Samman, 1972, 1976; Lutzner *et al.*, 1975; Sanchez & Ackerman, 1979). Recently, Guccion *et al.* (1979) proposed criteria for electron microscopic (EM) diagnosis of MF based on clustering of atypical lymphocytes (CMC) in the skin infiltrates. However, these EM criteria lead to a high false positive rate (25%) in benign conditions.

During the last few years our group has introduced two additional methods for the diagnosis of early CTCL lesions: (1) cytophotometric DNA measurements on Feulgen stained imprints from skin biopsies (Van Vloten, Van Duijn & Schaberg, 1974; Van Vloten, Schaberg & Van der Ploeg, 1977); (2) morphometric determination of the degree of nuclear indentation of the lymphoid cells on electronmicrographs of skin biopsies (Meijer *et al.*, 1979, 1980).

Of the two methods, DNA cytophotometry has been studied most extensively (Van Vloten *et al.*, 1974; Bosman & Van Vloten, 1976; Hagedoorn & Kiefer, 1977; Van Vloten *et al.*, 1977). The presence of cells with polyploid and aneuploid DNA values in skin imprint preparations is considered to be a strong indication of MF involvement. So far, false positive results have not been obtained with this method. The false negative rate is 9% (Van Vloten *et al.*, 1977).

Morphometric analysis showed that the CMC population in the CTCL lesions has a higher degree of nuclear indentation as expressed by the nuclear contour index (NCI = perimeter divided by the square root of the area) than have CMC in chronic benign skin lesions (McNutt, 1980; Meijer *et al.*, 1980). Moreover, CMC with nuclear contour indices over 11·5 were found exclusively in CTCL, although such high values do not occur in all patients with CTCL. On the basis of the morphometric measurements, all suspect cases, which developed CTCL during clinical and histological follow-up, could be classified with a high degree of probability (Meijer *et al.*, 1980).

The purpose of the present study is: (1) to evaluate the diagnostic reliability of the morphometric method for a total number of sixty-three patients (twenty patients with CTCL, fourteen patients with chronic benign skin diseases and twenty-nine patients suspected of CTCL with a maximal follow-up of four years); (2) to investigate whether the initial classification based on the NCI histograms can be further improved.

The results are compared with those obtained from cytophotometric DNA measurements of skin imprint preparations and are referred to the histological data and clinical follow-up. It is concluded that NCI measurements of lymphoid cells in the skin are of diagnostic value in the differential diagnosis between CTCL and chronic benign skin diseases.

MATERIAL AND METHODS

Patients

Three groups of patients were included in this study. The relevant clinical and histological data are presented in Tables 1, 2, and 3. The first group (group I) consisted of twenty patients (five females, fifteen males, age 38–82 years) with clinically and histologically diagnosed CTCL (Table 1). The second group (group II) was composed of fourteen patients (six females, eight males, age 18–69 years) with chronic benign skin diseases (Table 2). The third group (group III) consisted of twenty-nine patients (fifteen females, fourteen males, age 32–73 years) with skin lesions clinically and/or histologically suspected of being CTCL (Table 3). Two patients of group III (no. 131 and 132) showed the clinical picture of parapsoriasis en plaques and six patients of group III had erythroderma (no. 61, 135, 100, 116, 121 and 129).

The histological diagnoses 'suspected of MF or SS' were made by two pathologists (E.S. and C.M.). The maximum follow-up period of the suspect cases after the biopsy had been taken was four years.

TABLE 1. Correspondence between the nuclear contour index distributions and DNA cytophotometry in cutaneous T cell lymphomas

	Malignant training set (n = 20)						
Patient no.	Highest NCI value	NCI* 25	NCI* 70	Probability of classification† malignant	DNA‡	Follow-up (years)	Diagnosis (stage)
1	12·6	6·0	8·2	100%	+	2 died	SS
2	18·2	5·4	8·2	100%	+	2·5	SS
5	11·7	4·7	6·9	100%	+	2 died	SS
13	18·5	5·8	8·7	100%	+	3	SS
4	11·7	4·4	6·5	99·9%	+	1·5 died	MF III^{c}
7	11·9	5·2	7·1	100%	+	1 died	MF III^{c}
63	11·1	4·2	6·2	71%	n.d.	1·5	MF III^{c}
65	11·9	4·8	6·8	100%	+	2	MF III^{c}
107	10·2	4·5	6·0	95%	+	1	MF III^{c}
122	12·2	3·9	6·9	100%	+	0·3	MF III^{c}
6	15·5	4·6	6·4	100%	—	2·5	MF III^{b}
8	16·2	6·3	9·5	100%	+	2 died	MF III^{b}
108	12·3	5·1	6·8	100%	—	0·5	MF III^{b}
114	12·6	4·2	6·1	50%	+	2	MF III^{b}
39	12·0	5·0	6·6	100%	—	2	MF III^{b}
37	12·5	4·3	6·2	66%	+	2·5	MF III^{b}
17	12·8	5·1	7·2	100%	—	3	MF II^{b}
56	10·7	4·5	6·0	95%	+	2	MF II^{b}
3	10·2	4·4	7·1	100%	n.d.	5	MF II^{a}
10	10·6	5·2	7·1	100%	+	4	MF II^{a}

* The 25th and 70th percentiles of NCI distributions are those NCI under which 25 and 70% of all measured cells are classified.

† The number expresses the probability of classification (in percentages) in the malignant group according to the Alloc program using the 25th and 70th percentiles of NCI distributions as parameters.

‡ DNA distribution is considered to be abnormal (+) when hypertetraploid values are found. n.d. = Not done.

SS = Sézary syndrome.

MF = Mycosis fungoides.

The patients were staged according to the modified Fuks classification (Fuks, Bagshaw & Farber, 1973):

Stage I: Mycosis fungoides of the skin without palpable lymph nodes.

Stage II: Mycosis fungoides of the skin with palpable lymph nodes, showing the histological picture of dermatopathic lymphadenopathy without MF involvement.

Stage III: Mycosis fungoides of the skin with palpable lymph nodes, histologically showing MF involvement.

Stage IV: Mycosis fungoides of the skin with visceral involvement.

Histologically MF involvement of lymph nodes was determined according to the criteria of Scheffer, Meijer & Van Vloten (1980). The type of skin lesion was indicated as eczematous (a), plaque (b), tumour (c) and erythroderma (d).

To evaluate the specificity of lymphoid cells with NCI values $\geqslant 11{\cdot}5$ for CTCL, we examined the lymphoid cells in several benign conditions including peripheral blood of healthy donors (n = 6)

TABLE 2. Correspondence between the nuclear contour index distributions and DNA cytophotometry in chronic benign skin diseases

	Benign training set (n = 14)					
Patient no.	Highest NCI value	NCI*		Probability of classification malignant†	DNA‡	Diagnosis
		25	70			
14	9·6	3·8	4·4	0%	n.d.	Contact dermatitis
15	10·0	4·1	5·2	0·1%	n.d.	Contact dermatitis
33	10·9	4·2	5·3	0·4%	—	Contact dermatitis
41	8·5	3·9	4·6	0%	—	Chronic eczema
44	9·9	3·9	5·1	0%	—	Chronic eczema
46	10·6	4·2	5·7	17%	—	Chronic eczema
29	11·2	4·3	5·8	37%	n.d.	Atopic dermatitis
43	8·8	4·2	5·4	0%	n.d.	Jessner's disease
75	5·1	3·7	4·1	0%	n.d.	Jessner's disease
73	8·2	4·1	5·3	0%	n.d.	Lichen planus
34	6·5	3·8	4·1	0%	—	Lymphomatoid reaction
76	6·6	3·8	4·5	0%	—	Pyoderma gangrenosum
57	6·8	3·9	4·8	0%	—	Pseudolymphoma (Spiegler-Fendt)
67	8·3	3·9	4·7	0%	—	Pseudolymphoma (Spiegler-Fendt)

See Table 1 for explanation of abbreviations and symbols.

and patients with erythroderma supervening on psoriasis (n = 2) or atopic dermatitis (n = 1), human cord blood (n = 5), synovial fluid lymphocytes from patients with rheumatoid arthritis (n = 4), 4 days phytohaemagglutinin (PHA) stimulated blood lymphocytes (n = 3) and lymphocytes in lymph node cell suspensions from patients with unrelated diseases histologically showing dermatopathic lymphadenopathy (n = 6).

Specimen processing

Five millimetre punch skin biopsies were cut into two parts. From the freshly cut surface, imprint preparations were made for DNA cytophotometry (Van Vloten *et al.*, 1974). One part of the skin biopsy was fixed in 4% buffered formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin, Giemsa and perodic acid-Schiff (PAS) for routine histological examination. The other part was cut into small pieces and fixed in 2% cacodylate-buffered glutaraldehyde for 1 h and post-fixed for 2 h in 2% cacodylate-buffered osmium tetroxide. After dehydration the specimens were embedded in Epon 812. Ultra-thin sections were stained with uranyl acetate and lead citrate.

Cell suspensions containing mononuclear cells of the control specimens were embedded for electronmicroscopy as described previously (Meijer *et al.*, 1977). To avoid double sectioning of cells, ultra-thin sections were cut at 25 μm intervals and the whole grid was photographed at a magnification of 2000 × . From every skin biopsy and control specimen ten to twenty electromicrographs, each with approximately eight lymphoid cells, were prepared.

Morphometric analysis

Morphometric analysis of lymphoid cells was performed as described previously (Meijer *et al.*, 1980). Briefly, of all lymphoid cells present in electronmicrographs made of the skin biopsies and the control

TABLE 3. Correspondence between the nuclear contour index distribution and DNA cytophotometry in cases suspect for cutaneous T cell lymphomas

	Test set (n = 29)						
Patient no.	Highest NCI value	NCI* 25	NCI* 70	Probability of classification malignant†	DNA‡	Follow-up (years)	Diagnosis in follow-up
							Benign (n = 8)
61	9·7	4·2	5·7	17%	n.d.	0·5	erythroderma (psoriasis)
135	10·2	4·2	5·4	0%	n.d.	0·5	erythroderma (atopic dermatitis)
100	9·8	4·2	5·3	0·4%	—	1	erythroderma (pityriasis rubra pilaris)
125	10·3	3·9	4·5	0%	—	0·5	insect-bite
121	9·5	3·9	4·7	0%	—	0·5	erythroderma (atopic dermatitis)
115	7·7	4·1	4·8	0%	n.d.	1	Jessner's disease
127	7·5	3·8	4·1	0%	—	4	Jessner's disease
110	7·6	3·8	4·5	0%	—	1·5	Jessner's disease
							Lymphomatoid papulosis (n = 2)
47	10·8	4·8	7·1	100%	+	4	Lymph. pap.
130	10·6	4·1	6·8	100%	+	4 died	Lymph. pap.
							Still suspect for MF (n = 1)
32	13·0	4·7	6·0	100%	—	3	susp. MF
							MF (stage; n = 18)
126	14·7	5·0	7·5	100%	n.d.	5	MF I^{a}
105	13·9	4·8	6·7	100%	+	4·5	MF I^{a}
123	9·4	4·6	6·0	100%	+	0·5	MF I^{a}
124	10·4	5·3	8·0	100%	—	0·5	MF I^{a}
132	12·8	4·6	6·9	100%	+	0·1	MF I^{a}
131	10·8	4·4	5·7	40%	—	0·1	MF I^{a}
89	11·8	4·3	6·0	35%	—	1	MF I^{a}
74	11·9	4·9	6·4	100%	+	1·5	MF I^{b}
109	9·9	4·6	6·6	100%	n.d.	0·5	MF I^{b}
118	13·8	5·4	8·4	100%	+	0·5	MF I^{b}
111	12·4	4·1	5·2	0·1%	+	0·5	MF I^{b}
119	11·9	5·1	7·5	100%	—	0·5 died	MF I^{c}
116	13·0	4·5	6·4	100%	—	0·5	MF I^{d}
129	11·8	5·8	7·4	100%	—	0·1	MF I^{d}
112	10·8	4·3	7·0	100%	—	2	MF II^{b}
16	10·8	5·0	7·1	100%	+	5	MF III^{a}
71	11·0	4·8	6·5	100%	—	1·5	MF III^{c}
64	10·8	5·3	7·1	100%	+	2	MF III^{c}

See Table 1 for explanation of abbreviations and symbols.

specimens, the nuclear perimeter and area were measured with a graphic tablet (Tektronix) (Cornelisse *et al.*, 1980). At least seventy-five lymphoid cells, mostly 75–110 lymphoid cells, were measured. Mononuclear phagocytes were differentiated from lymphoid cells by the presence of organelle-rich cytoplasm with numerous azurophilic granules and lysosomes or lysosome-like structures, and a relatively low nucleus to cytoplasm ratio. From the nuclear area and perimeter, the nuclear contour index (NCI) ($= \frac{\text{perimeter}}{\text{area}^{\frac{1}{2}}}$) was calculated. The reproducibility of the NCI measurements is better than 90% (Meijer *et al.*, 1980). Morphometrically a lymphocyte is defined as a CMC when it has a NCI value ≥6·5.

Statistical analysis

Differences in the nuclear contour index histograms between the malignant and benign lesions were studied with the aid of discriminant analysis. A non-linear discriminant analysis method (Alloc-3) was used. This method does not assume multivariate normal distributions nor equality of covariance matrices within the two groups (Habbema & Hermans, 1977). Stepwise selection of histogram variables to be included in the discriminant function was done on a group of twenty histograms from patients with unequivocal CTCL (Table 1) and fourteen histograms from a second group of patients with chronic benign skin diseases (Table 2). The discriminant function derived from the most discriminatory histogram variables was used to classify the individual cases of the first two groups and the cases of the third group of twenty-nine patients suspected of CTCL (Table 3).

DNA cytophotometry

In order to compare the results of morphometric analysis with those based on the DNA measurements, DNA cytophotometry was performed on Feulgen stained skin imprint preparations of the fresh cut surface, as described by Van Vloten *et al.* (1974, 1977).

RESULTS

NCI threshold

Figure 1 shows typical NCI histograms from skin biopsies from patients with CTCL, chronic benign skin lesions and patients suspected of having CTCL.

Fifteen out of twenty patients with clinically and histologically diagnosed CTCL had lymphoid cells with NCI values larger than 11·5 (group I; Table 1). In the skin biopsies from patients with chronic benign skin lesions (group II; Table 2), lymphoid cells with NCI values ≥11·5 could not be found. The highest NCI value found in chronic benign skin lesions was 11·2 (no. 29; Table 2).

In all other control specimens examined no lymphoid cells were present with NCI value ≥11·5 (Table 4). The highest NCI value (10·8) was found in PHA stimulated peripheral blood lymphocytes (Table 4). Lymphoid cells with NCI values ≥11·5 were also present in the material from eleven patients of group III (suspected of CTCL; Table 3). In the follow-up period ten cases were diagnosed as CTCL, whereas one is still suspect for MF.

Compilation of the data of group I and the eighteen patients of group III, who developed MF during the follow-up period, indicates a sensitivity of 66% (25 out of 38) for the presence of cells with NCI values ≥11·5 as a diagnostic criterion for CTCL. Such cells are not present in skin lesions of patients with chronic benign skin diseases.

Discriminant analysis

To increase the sensitivity of the discrimination, we looked for other differences in the NCI histograms of the patients of group I and group II. A group of twenty patients with CTCL (group I; Table 1) and fourteen patients with chronic benign skin diseases (group II; Table 2) were used as a training set for the stepwise selection of the best discriminating NCI histogram variables. The following variables were selected by the Alloc-3 program: P25 and P70, being the 25th and 70th percentiles of the NCI histograms. A scatterdiagram of these two variables is shown in Fig. 2, in

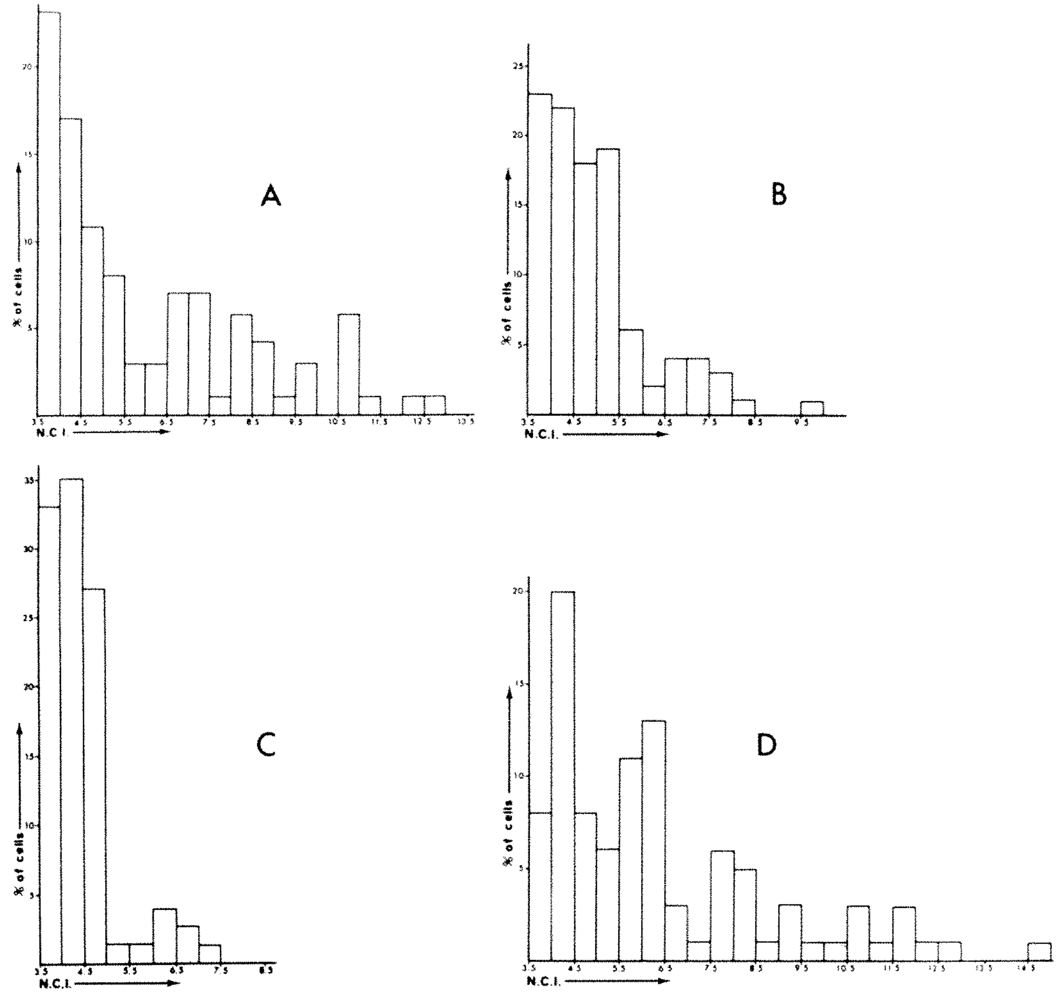

FIGURE 1. Nuclear contour index histograms of lymphoid cells in: (A) a patient with MF (no. 122; group I); (B) a patient with chronic eczema (no. 44; group II); (C) a suspect case, classified benign, in follow-up Jessner's disease (no. 110; group III); (D) a suspect case, classified as MF, in follow-up MF (no. 126; group III).

which two distinct clusters can be distinguished, the malignant and the benign group, with a small transitional area.

The discriminant function derived from these two variables, which will be denoted as P25 + P70, was tested on the same set and classified seventeen out of twenty CTCL patients with a probability of over 95% as malignant.* One patient (no. 63), who has skin tumours, was allocated with a probability of 71% and two others (nos. 37 and 114) with probabilities of 66% and 50%, respectively. From the benign cases (group II) twelve out of fourteen were classified as benign with probabilities over 95%, whereas two were classified as benign with lower probabilities, i.e. of 83% and 63%, respectively.

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* The estimated predictive values for benign or malignant are complementary and range from 0% to 100%. Thus a probability (predictive value) of 95% in the malignant class corresponds with a probability of 5% in the benign class.

TABLE 4. Maximum nuclear contour index values of CMC found in control specimens other than chronic benign skin lesions

Control specimens	Range of maximum NCI value in control specimens
Healthy blood donors (n = 6)	7·5–10·2
Human cord blood (n = 5)	8·0– 8·7
PHA stimulated blood lymphocytes (n = 3)	10·2–10·8
Rheumatoid synovial fluid (n = 4)	8·2– 9·7
Blood from patients with chronic benign skin diseases (n = 3)	8·0–10·0
Dermatopathic lymph nodes from non-MF patients (n = 6)	7·5–10·5

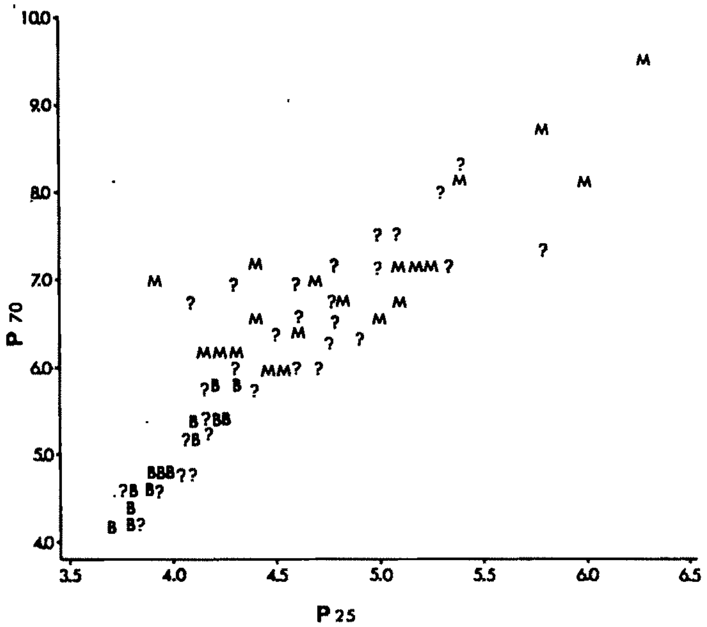

FIGURE 2. Scatter diagram of the 25th versus the 70th percentiles of NCI distributions of malignant cases (M), benign cases (B) and suspect cases (?). P25 and P70: the 25th and 70th percentiles of the NCI histograms.

The diagnostic value of the discriminant function was then tested on an independent set of cases (test set = group III) suspected of CTCL as specified in Table 3. Eleven out of twenty-nine cases were classified as benign, eight of which with probabilities over 95% and three with probabilities of 83%, 65% and 60%, respectively. One of the patients classified as benign with a probability over 95% (no. 111) and two classified as benign with probabilities of 60% and 65% (nos. 131 and 89) developed CTCL during the follow-up period. Eighteen patients were classified as malignant with probabilities over 95%, from which fifteen developed CTCL during the follow-up period. Two patients appeared to have lymphomatoid papulosis and one is still suspect for CTCL in the follow-up period (Table III).

For the total material (group I and eighteen cases of group III), thirty-two out of thirty-eight patients with early or advanced CTCL lesions were classified correctly when a probability ≥95% for malignancy is taken as a decision criterion for definite CTCL involvement.

Thus it appears that the discriminant function derived from the NCI histogram variables has a considerably higher sensitivity than the NCI threshold value ≥11·5 (84% versus 66%).

Combination of the two classification rules (P25+P70; NCI threshold ≥11·5) gives the following results (Table 5): If one or both criteria indicate malignancy, thirty-six out of thirty-eight cases (group I and those cases from group III which in follow-up proved to be CTCL) are classified correctly. One in follow-up is still a suspect case and two cases of lymphomatoid papulosis are also classified as malignant. Two patients are classified as inconclusive (nos 63 and 131; Probability <95%). It appears that all twenty-two benign cases are correctly classified.

DNA cytophotometry

The results of DNA cytophotometry are shown in Tables 1, 2 and 3. On the skin biopsies of thirty-four patients with early or advanced CTCL (eighteen patients of group 1 and sixteen patients of group III), DNA cytophotometry was performed. Twenty-two out of thirty-four patients (fourteen patients of group I and eight patients of group III) with CTCL had abnormal DNA histograms.* Normal DNA histograms were found in twelve out of thirty-four patients with CTCL (four patients of group I and eight patients of group III; false negative rate: 35%). Six out of twelve CTCL patients with normal DNA values were in the early stages of MF (nos 124, 131, 89, 119, 116 and 129). The DNA histogram of the patient still suspect for MF (no. 32; Table 3) showed a normal DNA histogram.

Abnormal DNA histograms were found in both cases of lymphomatoid papulosis (nos 47 and 130; Table 3).

In eight patients with chronic benign skin diseases (group II) and five patients originally suspect for MF but in follow-up proved to be of benign origin, DNA cytophotometry of infiltrated cells was performed. All patients had normal DNA histograms.

Four CTCL cases (nos 6, 108, 39 and 17; group I) and seven cases originally suspected of CTCL and confirmed in follow-up (nos 124, 89, 119, 116, 129, 112 and 71; group III), in which DNA cytophotometry was negative, were classified correctly by morphometric analysis (P25+P70 or NCI threshold ≥11·5).

DISCUSSION

The results of the present study show that indicator cells, i.e. lymphoid cells with NCI values ≥11·5, are found only in the skin lesions of patients with CTCL. Apart from the occurrence of these indicator cells, the frequency distribution of lymphoid cells with NCI values larger than 6·5 is increased in CTCL lesions (P25+P70) as compared to skin biopsies from patients with chronic benign skin lesions. By combining both criteria it was possible not only to classify correctly patients with CTCL and chronic benign skin lesions with a high degree of sensitivity and specificity, but also to classify patients suspected of CTCL in the correct group as was proven by follow-up. These results thus confirm earlier findings reported in a preliminary study (Meijer *et al.*, 1980).

We evaluated the specificity of CMC with NCI values of 11·5 or more by measuring the NCI values of CMC in several benign conditions, i.e. in peripheral blood of neonates, healthy donors and patients with benign erythroderma, in synovial fluid of patients with rheumatoid arthritis and in chronic benign skin lesions. Since CMC are numerous in lymph nodes with dermatopathic lymphadenopathy (Rausch, Kaiserling & Goos, 1977; Scheffer *et al.*, 1980), we measured CMC in lymph

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* Cells with abnormal or hypertetraploid DNA values are cells with a DNA content of more than 4n+2s.d.

TABLE 5. Specificity and sensitivity of different criteria for the morphometric classification of cutaneous T cell lymphomas as compared to chronic benign skin lesions

	Morphometric criteria								DNA cytophotometry	
	NCI $\geqslant$ 11·5* Morphometric classification		P25 + P70 with a probability $\geqslant$ 95%† Morphometric classification			NCI $\geqslant$ 11·5 or P25 + P70 with a probability $\geqslant$ 95% Morphometric classification			Normal DNA values DNA classification	Abnormal DNA values
Histological and clinical diagnosis after follow-up	Benign	CTCL	Benign‡	CTCL‡	Inconclusive§	Benign‡	CTCL‡	Inconclusive§	Benign	CTCL
Benign (n = 22)	22	—	19	—	3	22	—	—	13	—
Still suspect for mycosis fungoides (n = 1)	—	1	—	1	—	—	1	—	1	—
Cutaneous T cell lymphomas (n = 38)	12	25	1	32	5	—	36	2	12	22
Lymphomatoid papulosis (n = 2)	2	—	—	2	—	—	2	—	—	2

* Criterion: presence of indicator cells, i.e. cells with nuclear contour index values of 11·5 or more.

† Criterion: frequency distribution of cerebriform mononuclear cells in the skin infiltrates, expressed by the 25th and 70th percentile (P25 + P70) of the NCI historgram.

‡ Benign or malignant (CTCL): classification in this group with a probability $\geqslant$ 95%.

§ Inconclusive: classification with a probability lower than 95% benign or malignant.

Abnormal DNA values: the presence of cells with hypertetraploid DNA values (more than 4n + 2 × s.d.).

CTCL: cutaneous T cell lymphomas.

nodes with dermatopathic lymphadenopathy of patients with diseases unrelated to CTCL. Because it has been shown that CMC are activated T cells (Meijer *et al.*, 1977), we also measured the degree of nuclear indentation of peripheral blood lymphocytes stimulated *in vitro* by the T cell mitogen PHA. In all these conditions no CMC with NCI vales ⩾11·5 were found. Thus, CMC with a NCI value ⩾11·5 seem to be a specific marker for CTCL lesions, but of limited sensitivity (66%).

Because of the limited sensitivity of these indicator cells for classifying patients in the benign or malignant group, discriminant analysis was used to find differences in the NCI histograms of the skin infiltrates of benign and malignant lesions. Using the discriminant function it was possible to discriminate between malignant and benign skin lesions when as parameter the frequency distribution of CMC in the skin infiltrates was used, expressed as P25+P70 of the NCI histograms. With this discriminant function (P25+P70), five out of thirty-eight CTCL patients (nos 63, 114, 37, 131 and 89) were classified as inconclusive, one CTCL patient (no. 111) was classified benign. This results in a sensitivity of 84% to diagnose CTCL which is far more sensitive than the classification based on the presence of indicator cells.

If both classification rules (P25+P70; NCI threshold ⩾11·5) are combined, the sensitivity rises to 95%. This high sensitivity and specificity makes this method useful in the differential diagnosis between CTCL and chronic benign skin diseases.

One patient (no. 32) whose skin lesions were morphometrically classified malignant developed a persistent dermatitis 4 years ago, originally thought to be caused by gold injections given for 1 year for his rheumatoid arthritis. Because of suspicion of MF a skin biopsy was taken. The histology was suspected of MF. After the treatment was stopped, three years ago now, this patient still has minor skin lesions.

Of the two CTCL cases which were allocated as inconclusive by both classification rules (P25+P70 or NCI threshold ⩾11·5), one case (no. 63) was in the more advanced stage of MF (tumour), the other CTCL case (no. 131) was in the premycotic stage of the disease. Tumour lesions, however, may be a potential source of false negatives, for they may show decreased numbers of CMC (Braun-Falco, Schmoeckel & Wolff, 1977; Edelson, 1980; Meijer *et al.*, 1980), possibly by transformation into blast like cells (Braun-Falco *et al.*, 1977; Edelson, 1980). In this study, however, the remaining seven out of eight patients with skin tumours were classified correctly (nos 4, 7, 65, 107, 122, 71 and 64) with the two classification criteria. Histologically and clinically, however, MF patients with skin tumours are not difficult to diagnose. Therefore, in practice, this possible source of error will not be important, since this method will only be used for the diagnosis of the early stages of MF, which clinically and histologically are difficult to differentiate from chronic benign skin diseases (Samman, 1972, 1976; Lutzner *et al.*, 1975; Sanchez & Ackerman, 1979).

The results of DNA cytophotometry show that abnormal DNA values were found in the skin biopsies of twenty-two out of thirty-four patients with CTCL. None of the patients with chronic benign skin diseases, proven by follow-up, had abnormal DNA values. However, two patients with lymphomatoid papulosis were classified as malignant (see below). DNA cytophotometry proved to be less sensitive (65%) than the morphometric classification (P25+P70 or NCI threshold ⩾11·5). In eleven CTCL cases (nos 6, 108, 39, 17, 124, 116, 129, 112, 119, 89 and 71), DNA cytophotometry was negative whereas the morphometric classification allocated these cases correctly. For the discrepancy between DNA cytophotometry and the morphometric classification, three possible reasons can be distinguished: (1) no relationship exists between the abnormal DNA value and the degree of nuclear indentation; (2) abnormal DNA values appear later than an altered CMC frequency distribution and/or presence of indicator cells in morphometric measurements; (3) sampling errors may be involved, since DNA measurements and morphometric analysis are done on different cells i.e. imprint preparations (DNA cytophotometry) and skin biopsy material (morphometric analysis).

Moreover we did not use as a parameter for malignancy the frequency of pure tetraploid cells, because this frequency is influenced by the sampling method and number of cells measured. Flow-cytophotometry may improve this.

Two patients with lymphomatoid papulosis (nos 47 and 130) were classified malignant on the basis of the frequency distribution of CMC. Also, abnormal DNA values were found in the skin imprint preparations. This is not surprising since lymphomatoid papulosis histologically resembles CTCL, but in most cases clinically runs a benign course. Some authors consider this disease to belong to the spectrum of CTCL (Lutzner *et al.*, 1975). It may actually represent a kind of 'no man's land between an immunological reaction and lymphoma' (Wilson Jones, 1979).

In conclusion, the results of this study show:

(1) Morphometric differentiation between CTCL and chronic benign skin lesions is possible with a high specificity and sensitivity when the following parameters are used: the presence of indicator cells (lymphoid cells with NCI values $\geqslant 11{\cdot}5$) or the frequency distribution of CMC in the skin infiltrates (P25+P70; probability $\geqslant 95\%$).

(2) DNA cytophotometry is a useful additional criterion for the diagnosis of malignancy, i.e. CTCL, because the presence of cells with abnormal DNA values in skin infiltrates indicates CTCL involvement. However, it is a less sensitive way to diagnose CTCL than morphometric measurements.

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Flow cytometry as a tool for the study of cell kinetics in skin 2. Cell kinetic data in psoriasis

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SUMMARY

Flow cytometry was used to measure the DNA content of epidermal keratinocytes from psoriatic patients. Gross deviations were found in the lesions and minor but significant changes in the uninvolved skin. Statistical analysis revealed that the rather large variation in the DNA distributions of the lesions was due to inter-individual differences rather than to intra-individual differences. The duration of the S-phase seemed to be prolonged in the lesion, but the length of the overall cell cycle might be of the same order as that of normal skin.

Studies on cell cycle parameters in psoriasis have given rather conflicting findings (for reviews see Gelfant (1976) and Grove (1979)). These disagreements might be due to several factors. As to the lesion itself, the maturity of the lesion, size and location might play a role. Also inter-individual differences could be important and, last but not least, to a certain extent pitfalls in the methods can contribute to this unsatisfactory situation.

Flow cytometry (FCM) has proved to be a fast and accurate method for the analysis of the cell cycle (for a review see Gray & Coffino, 1979). We have used this method for the study of cell cycle parameters in normal skin (Bauer *et al.*, 1980) and report here on FCM measurements in psoriasis both in uninvolved skin and in the lesion.

MATERIALS AND METHODS

Biopsies were taken from lesions or from uninvolved skin of patients suffering from psoriasis vulgaris at their visit to the Outpatient Department. Those selected had not had therapy for at least 1 week prior to the biopsy. Sampling, preparation of single cell suspensions, measuring procedures and statistical analysis were carried out as described earlier (Bauer *et al.*, 1980).

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RESULTS

Psoriatic lesion: mean DNA distribution

Table 1 shows the DNA distributions of stable lesions in patients suffering from psoriasis vulgaris. 9·7% of the cells were in S-phase and 2·9% in G_2M. The ratio S/G_2M (standing for the relative duration of S to G_2M) was 4·1. The figures for normal skin were 2·7 for S and 1·3 for G_2M (Bauer *et al.*, 1980).

TABLE 1. Percentage DNA distribution of normal, non-involved psoriatic and psoriatic epidermis and standard deviations. The figures for normal epidermis are taken from Bauer *et al.* (1980)

		Normal epidermis (n = 60)	Non-involved psoriatic epidermis (n = 47)	Psoriatic lesion (n = 94)
Uncorrected values (see Baur *et al.*, 1980)	2c	95·9 ± 1·1	94·5 ± 2·0	87·4 ± 3·4
	S	2·7 ± 0·8	3·8 ± 1·4	9·7 ± 2·5
	G_2M	1·3 ± 0·6	1·8 ± 0·8	2·9 ± 1·4
	Ratio S/G_2M	2·6 ± 1·1	2·3 ± 0·8	4·1 ± 2·2

Compared to normal skin the variation between the individual specimens is rather large. This could arise from several factors; among others, from variation in the lesion itself, variation between different lesions from one person and from inter-individual differences. The share of each of them was investigated.

Inter-individual and intra-individual variance

Analysis of the cause of the variability is most conveniently done using variance rather than standard deviation (note that the latter is presented in Table 1).

The variances for psoriatic lesions are derived from a population of ninety-four samples from seventeen patients. The question arises whether this population is homogeneous as to the contributing patients, in other words whether the variance in the subpopulation of biopsies from one patient is of the same order as or is significantly smaller than the variance in the total population. Therefore we performed a one-way variance analysis with the factor patient number. The variance is divided into an inter- and an intra-individual component (Table 2).

It can be concluded from Table 2 that the individual subpopulations are significantly more homogeneous than the total population. Minimally 39% and maximally 64% of the total variance is explained by inter-individual differences. The number of biopsies and the sample area were not identical for all patients. A two-way analysis as to site and patient number gave no significant increase of the explained variance.

Intra-lesional variance

In order to investigate whether a lesion was kinetically homogeneous, we took a total of thirty-one biopsies gathered from the centre and the rim of six lesions according to Table 3. The rim is defined as the outer 1·5 cm of the lesion, the centre as the area enclosed by the rim.

TABLE 2. Analysis of the variance in inter- and intra-individual contributions (for references see program 'Anova', N.I.E., 1975; U.R.C., 1979)

	2c*	S*	G_2M*	Ratio*
Inter-individual variance	6·6	4·8	2·6	3·3
Intra-individual variance	2·2	1·8	1·0	1·9
Explained variance	64%	61%	57%	39%

* $P < 0·01$.

For this study the biopsies from each individual were taken from one lesion. The site of the lesion was not identical for each patient. The significance of possible differences of the mean values of each patient between rim and centre is assessed by the Student's '*t*'-test considering that the samples are paired as to the patient. It is clear from Table 4 that no significance between rim and centre could be detected.

Uninvolved skin

Table 1 shows the DNA distribution in the non-involved skin of patients. The percentage of cells in S is 3·8; of G_2M 1·8. The ratio S/G_2M is 2·3. The percentage S is significantly higher than in normal

TABLE 3. Summary of the biopsy sites of each patient

Patient no.	Rim			Centre		
	Leg	Back	Abdomen	Leg	Back	Abdomen
1		2			1	
2		2			1	
3			4			3
4		4			5	
5	2			3		
6			2			2
Total	2	8	6	3	7	5

TABLE 4. Student's '*t*'-test on the paired difference of the mean values of centre and rim in 6 patients

		2c*		S*		G_2M*		Ratio*	
	n	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Difference centre-rim	6	0·04	3·3	−0·61	2·8	0·58	1·0	−0·42	1·5

* $P > 0·1$.

skin ($P<0{\cdot}1\%$) as is the percentage G_2M ($P<0{\cdot}1\%$) (tested with the Student's '*t*'-test). The ratio S/G_2M shows no significant difference.

DISCUSSION

Gross deviations in DNA distribution are found in the number of cells in the S-phase and in G_2M in the psoriatic lesion. As these percentages of cells in S and G_2M refer to the total cell number and not to the proliferative cells these percentages are not directly comparable to the labelling index (LI) as derived from autoradiographic studies.

We could show in this study that the considerable variance in the percentages 2c, S and G_2M was due mainly to inter-individual differences (Table 2). Whether these differences are caused by differences in the cell cycle itself or are due to changes in the ratio non-cycling cells to cycling cells in different subjects suffering from psoriasis vulgaris has yet to be demonstrated. Nevertheless, this finding might perhaps explain the clinical observation that the response to therapy can be so different in subjects that are clinically alike. In the words of Mali (1979), it seems that 'everybody has his own psoriasis'.

What we do find in our experiments is a shift in the ratio S/G_2M in psoriasis to higher values than in normal skin.

Comparison of our present findings with the existing literature is of limited value because of the diversity of previous views. The review of Gelfant (1976) deals with this matter in detail, and it is clear that an independent technique such as flow cytometry could be of great value. In particular, our observations support the autoradiographic findings of Born & Kalkoff (1969) and Pullmann, Lennartz & Steigleder (1977) that the duration of the S-phase is prolonged in the psoriatic lesion, although other explanations are possible, for instance a shortening of G_2M. There are, however, no data about changes in G_2M while on the other hand prolongation of S is found in premalignant lesions (Heenen *et al.*, 1975).

The relatively small changes in the ratio S/G_2M seem to conflict with the findings of Goodwin, Hamilton & Fry. (1973) who studied the ratio of labelled cells to mitotic cells. These workers found 25:1 in clinical psoriasis, 190:1 in uninvolved psoriatic epidermis and 450:1 in normal epidermis. If we assume that the labelled cells are cells in the S-phase it would mean that the duration of S to the duration of mitosis is normally 450:1. Assuming an S-phase duration of 8 h the duration of mitosis would become about 1 minute. It is more reasonable therefore to assume that the labelled cells for the greater part reflect what has been termed 'metabolic DNA', a suggestion already made by these authors. Flow cytometry, on the other hand, measures DNA content and a cell with a DNA content between 2c and 4c will therefore likely be in S-phase.

A further point that could explain the obvious differences is that during the preparation procedure with the proteolytic enzyme pepsin, cells in mitosis are destroyed (Beck, 1977). Probably the same is true for trypsin. The percentages of 4c cells then reflect G_2 rather than G_2M. Changes in the duration of mitosis therefore will not be observed.

No statistical difference was found in DNA distribution within the lesion itself (Table 4). The finding of Soltani & Van Scott (1972), who reported an upward shift in the mitotic index from the centre of the lesion to the periphery, is not necessarily in contradiction with our finding if we suppose that in our procedure mitoses are destroyed. The differences in MI index would then reflect a prolongation of T_m in the periphery of the lesion.

As we pointed out before, the percentage S or G_2M from FCM measurements can be used for the calculation of the turnover time of the viable epidermis if the duration of the S or G_2M phase is known (Bauer *et al.*, 1980).

Using our present values of 4·1 and 2·6 for the ratios S/G_2M in psoriatic lesion and normal skin (Table 1), and taking T_s in normal skin to be 8 h (reviewed by Gelfant, 1976), we may calculate that

$$T_s\ (\text{lesion}) = \frac{4{\cdot}1}{2{\cdot}6} \times 8 = \pm 13\ \text{h}$$

The turnover time for the lesion is then given by

$$T_{\text{turne.p.}} = \frac{T_s}{\%S} \times 100$$

$$= \frac{13}{9{\cdot}7} \times 100 = 134\ \text{h or } 5{\cdot}5\ \text{days.}$$

In this calculation we suppose that the change in the ratio is due to a prolongation of the S-phase duration, while T_{G_2M} is unchanged. The figures we find are roughly in agreement with the estimation by Van Scott & Ekel (1963), derived from morphometric measurements and mitotic counts. The cell cycle time (T_c) should become about half of this value, i.e. about 67 h if we suppose that the proportion of cycling cells to differentiated cells is about 1:1 (Weinstein & Housman, 1972). This is of course a rather crude estimate and individual differences may occur as can be seen from the standard deviation in %S.

For psoriatic skin it is generally accepted that all proliferative cells are indeed in cycle. For normal skin, however, there are serious doubts (Gelfant, 1976). Our estimate of T_c for normal skin of 131 h (Bauer *et al.*, 1980) was based on the assumption that all proliferative cells were cycling, so T_c is the apparent cell cycle time. If only part of them are cycling, the cell cycle time becomes shorter. In fact the cell cycle time might be not so different at all in psoriasis. The abnormal cell production then has to be explained entirely in terms of the number of cycling cells per unit surface area, a suggestion already made by Chopra & Flaxman (1974) from *in vitro* studies.

From this point of view the study of Heenen, de Martelaer & Galand (1979) is of particular interest. These workers carried out computer simulation studies, and concluded that a transitory shift in the normal balance between the rate of cell flow to the differentiated and that to the proliferative compartment is sufficient to cause a permanent increase in the germinative population; thus without a change in the duration of the cell cycle time itself, a psoriatic alteration in epidermal morphology could be simulated. Our findings seem to support this hypothesis, although minor changes in the duration of the separate phases do exist (prolonged S-phase). In the light of these findings the concept of Gelfant (1976; 1978) that the treatment of psoriasis should concentrate on efforts to prevent G_0-cells going into cycle deserves attention.

The finding that the percentages of S and G_2M in uninvolved psoriatic skin differ significantly from normal skin can be explained in several ways. In the light of the above mentioned finding that there might be no large differences in cell cycle time the rise in S and G_2M could be explained by the fact that in uninvolved psoriatic skin the ratio non-cycling cells to cycling cells changed in favour of the latter. Following an appropriate stimulus this ratio might change further and give rise to a clinical lesion.

The ratio S/G_2M in the uninvolved skin did not change compared to normal skin. This seems not to support the findings of Steigleder & Pullmann (1979) that T_s in uninvolved psoriatic skin is prolonged.

Current research will concentrate on multiple-parameter FCM measurements that make it possible to discern between differentiated keratinocytes and proliferative cells, and on FCM stathmokinetic studies in order to obtain absolute cell cycle times.

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Autoradiographic investigations on the cell kinetics of epidermis and periderm of limb buds from mouse embryos *in vitro*[*]

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SUMMARY

Upper limb buds of mouse embryos (day 11+3 h of development) were cultured for 6 days. During this time the epidermis develops from a two-layered stage, consisting of a basal cell layer and a periderm cell layer, to a multilayered squamous epithelium with a stratum granulosum and a stratum corneum. To investigate the cell kinetics of epidermis and periderm during epidermogenesis the limb buds were labelled with ^{3}H-thymidine at different stages of development. The migration of labelled cells was studied on day 3 *in vitro*. In the first period of development, before a stratum granulosum has differentiated, each individual cell layer of the epidermis has a cell cycle of its own, i.e. once it has developed each cell layer grows independent of the other. The switching from horizontal to vertical proliferation starts on day 4 of culture with the appearance of the stratum granulosum and is completed on day 5 when a corneal layer begins to develop. With the appearance of the stratum corneum the limb bud shows the typical proliferation of the adult epidermis, which is regenerating only from the basal layer.

The labelling behaviour of periderm cells also shows that these cells have a cell cycle of their own and are not formed by cells migrating from the epidermis in an upward direction.

The first step of embryonic epidermogenesis is the differentiation of the unspecific one-layered ectoderm into a cuboid basal cell layer and a flat external periderm layer. In the limb bud of the mouse this differentiation begins on day 9·5 of development (Jurand, 1965). In a second step of epidermogenesis the cuboid basal cell layer changes into the typical multilayered cornified squamous epithelium. The other cell layer seen at the beginning of embryonic epidermogenesis, the periderm,

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is a special embryonic cell layer of the epidermis, which has no equivalent for the adult epidermis; It probably functions in the transfer of substances between embryo and amniotic fluid (Wolf, 1967; Hoyes, 1968) and acts as protection for the epidermis (Serri & Montagna, 1961). During the development of the stratum corneum the periderm cells lose their anchorage and are desquamated into the amniotic cavity (Hanson, 1947; Weiss & Zelickson, 1975).

Differences in growth and function between adult and embryonic epidermis suggest that proliferation and differentiation processes of the adult epiderm cells differ from those of embryonic epiderm cells. Proliferation of the adult epidermis takes place in the cylindrical epithelial cells of the basal layer (stratum basale). Epiderm cells originating from mitoses in the basal layer migrate via various differentiation stages (stratum granulosum, stratum corneum) to the skin surface where they are desquamated. Reports on proliferation processes in embryonic epidermis and periderm are still contradictory.

Some authors have observed mitotic activity in the periderm of human embryos (Breathnach & Robins, 1969; Breathnach, 1971). This finding would support the concept that the periderm has a cell cycle which is independent of the underlying epidermis. This finding is in disagreement with investigations in which no labelling of periderm cells was observed after addition of ^{3}H-thymidine (Gerstein, 1971). Although it may be possible that the varying statements about the cell cycle of periderm cells are due to differences in the time of investigation of the embryos (Holbrook & Odland, 1975), the final solution of the problem is still pending.

When the epidermis of 11-day-old chick embryos was treated with ^{3}H-thymidine, labelled cells could be detected in all layers of the epidermis, whereas two days later only the basal cell layer was labelled (Wessels, 1963). It could also be shown that during epidermogenesis, localization and orientation of mitoses changed in individual cells (Smart, 1970). These findings show that the cell kinetics during epidermogenesis will fundamentally change depending on the age of the embryo.

The aim of the present study is to investigate the cell kinetics of embryonic epidermis and periderm by autoradiographic means. Epidermogenesis was followed with the limb bud system established at our institute (Neubert, Merker & Tapken, 1974; Schultz-Ehrenburg, 1975; Herken, 1977) to answer the following questions:

(1) What are the differences between the cell kinetics of embryonic epidermogenesis and that of adult epidermis?

(2) Do the cell kinetics of embryonic epidermis change during its various differentiation stages?

(3) Does the embryonic periderm have a cell cycle of its own or are periderm cells formed by migration of epiderm cells from beneath?

MATERIALS AND METHODS

Upper limb buds of mouse embryos (strain NMRI) were cultured in an organ culture modified according to Aydelotte & Kochhar (1972). Details of the limb bud culture system have been described elsewhere (Neubert *et al.*, 1974; Herken, 1977). The upper limb buds of mouse embryos were removed under aseptic conditions on day 11+3 h and placed into the culture (day 11+3 h = 42 somite pairs). The day when sperm could be detected in vaginal smears at 8 a.m. was designated as day 0 of development.

For long-term labelling, three groups consisting of eight limb buds each were cultured for 1, 2 or 5 days in a normal medium and transferred to a medium containing 5 μCi/ml ^{3}H-thymidine (Amersham-Buchler, specific activity 5 Ci/mmol). Twenty-four h later the limb buds were removed from the radioactive medium and fixed in glutaraldehyde, modified according to Karnovsky (1965).

For pulse labelling, four groups consisting of four limb buds each were cultured for 3, 4, 5 or 6 days in a normal medium and placed for 1 h in a medium containing 5 μCi/ml ^{3}H-thymidine. Subsequently the limb buds were fixed as described above.

Since the development from the two-layered to the multilayered epidermis occurs about 3 days after beginning of the culture period (Schultz-Ehrenburg, 1975), the migration of epiderm cells during epidermogenesis was investigated at this stage and the 1-h pulse labelling with ^{3}H-thymidine was performed on day 3 of the culture period. Subsequently the limb buds were placed in unlabelled medium for further growth. At 1–2-h intervals up to 10 h after pulse labelling, groups of four limb buds each were removed from the medium and fixed.

All fixed limb buds were dehydrated in an acetone series and embedded in Mikropal (Ferak). 1 μm semi-thin sections were prepared and coated by dipping into an Ilford K5 photoemulsion. After exposure the sections were developed in Amidol and fixed with sodium thiosulphate. Then, the preparations were stained with alkaline Giemsa solution and embedded in Eukitt.

RESULTS

Light microscopy

After 2 days *in vitro* the limb bud epithelium consisted of two layers, a basal cell layer and the periderm. After 3 days the development of the epidermis had considerably progressed, especially in those regions where the limb buds were growing. In the region of the AER the epidermis consisted of up to six cell layers. However, there were also regions with only two cell layers, as seen on day 2. Cells of the newly formed intermediate layer were morphologically similar to those of the basal layer. In some areas the upper layers of the intermediate zone had changed from cuboid to flat epithelial cells.

After 4 days in culture, some areas of the most superficial cell layers of the epidermis showed keratohyaline granules, indicating that a stratum granulosum had started to develop. The number of cells, which in the superficial cell layer had changed into flat cells, had increased. Moreover, it was striking that in some regions of the basal layer the cuboid epithelial cells had assumed a cylindrical shape.

After 5 days *in vitro* in some areas the differentiation of the epidermis had progressed up to a stratum granulosum and partly also to a stratum corneum. It may be stated that as early as after 4 days, but to a larger extent after 5 days *in vitro*, skin differentiation proceeded in different degrees. In some areas the epidermis was, even after 5 days, similar to that after 3 days.

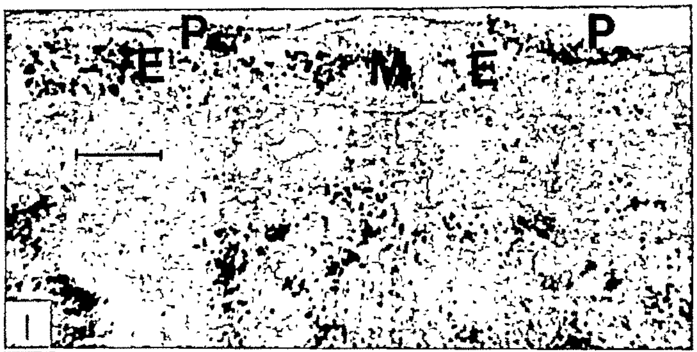
After 6 days the epidermis was mostly covered by a cornified layer. A continuous periderm layer could no longer be seen. A few flat cells resting on the stratum corneum could be taken as periderm cells but could not clearly be identified as such.

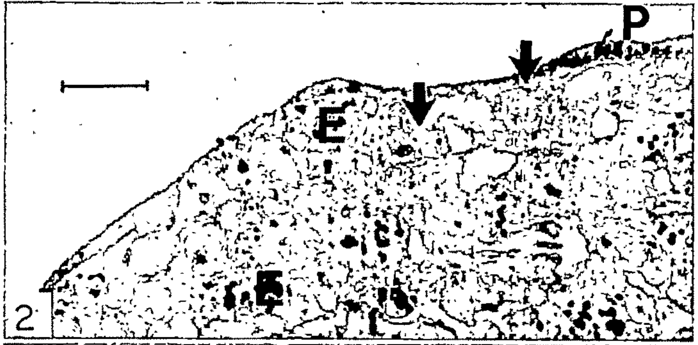
Autoradiography

Labelling for 24 *h*. In order to find out when the proliferation of the limb bud cells is strongest limb bud cells were labelled with ^{3}H-thymidine for 24 h on day 1, 2 and 5 and removed on day 2, 3 and 6 *in vitro*. After 2 days *in vitro*, almost all cells of the basal epidermis but only a few periderm cells were labelled (Fig. 1). After 3 days in culture, the labelling of basal cells was not as pronounced as after 2 days. Labelled cells were also detected in the newly formed intermediate zone of the epidermis. As after 2 days in culture, a few labelled periderm cells could be recognized. After 6 days *in vitro* the basal layer of the epidermis was labelled almost completely. It was striking that despite a labelling duration of 24 h labelled cells were not present in the other epiderm layers.

Labelling for 1 *h*. After 3 days *in vitro*, labelled cells appeared in all layers of the epidermis (Fig. 2)

In the AER labelled cells were even located on top of each other in a column-like fashion. Labelled cells occurred sporadically in the periderm. Unlabelled mitotic periderm cells could also be observed though they were rare. A few of the epithelial cells of the intermediate zone changed from cuboid to flat cells and were also labelled. Besides labelled cells, unlabelled mitoses were present in all layers of the epidermis.





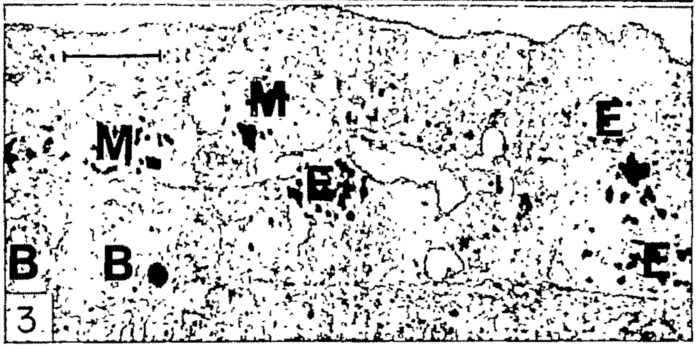
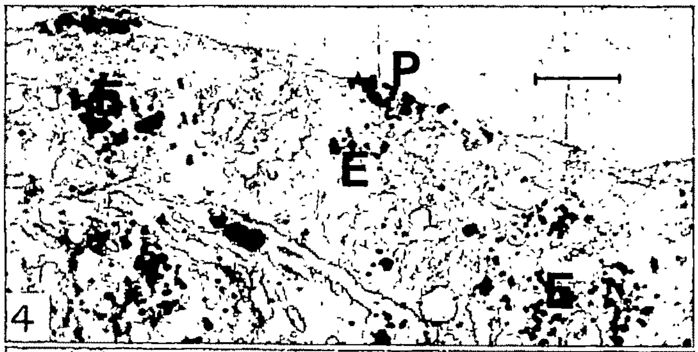
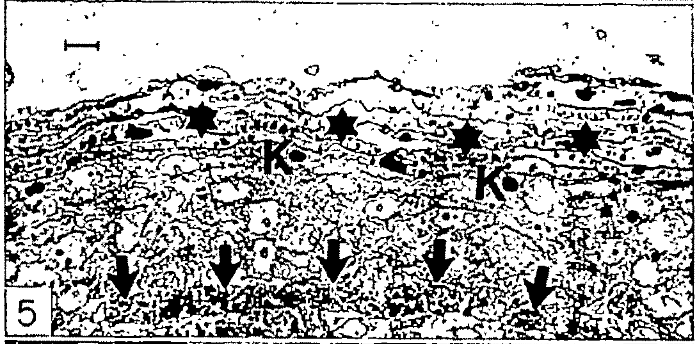


FIGURE 1. Section through the skin of an upper limb bud, obtained from a mouse embryo, day 11+3 h. 2 days *in vitro*. Labelling for 24 hours with ^{3}H-thymidine. P = labelled periderm cell, E = labelled epiderm cell, M = labelled mitosis of an epiderm cell. Scale bar = 10 μm.

FIGURE 2. Section through the skin of an upper limb bud as above, 3 days *in vitro*. Pulse labelling with ^{3}H-thymidine for 1 h. P = Labelled periderm cell, E = labelled epiderm cell, arrow = cells of the intermediate layer, which are flattened. Scale bar = 10 μm.

FIGURE 3. Section through the skin of an upper limb bud, 3 days *in vitro*. 10 h after pulse labelling with ^{3}H-thymidine for 1 h. B = Beginning change of the basal cells from cuboid to cylindrical cells, M = labelled mitoses, E = labelled epiderm cells. Scale bar = 10 μm.





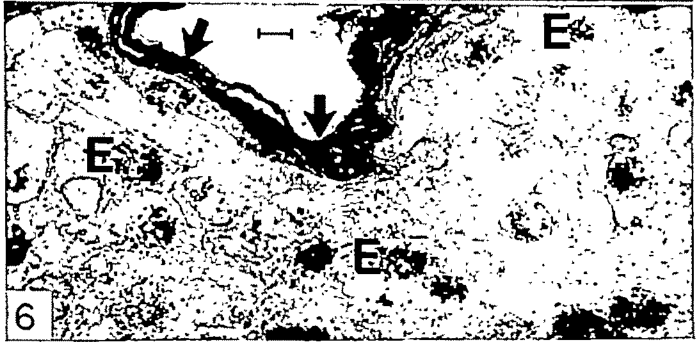


FIGURE 4. Section through the skin of an upper limb bud, 3 days *in vitro*. Four hours after pulse labelling with ^{3}H-thymidine for 1 h. P = Labelled periderm cell, E = labelled epiderm cell. Scale bar = 10 μm.

FIGURE 5. Section through the skin of an upper limb bud, 6 days *in vitro*. Pulse labelling for 1 h with ^{3}H-thymidine. Arrow = Labelled cells of the basal layer, K = keratohyaline granules (stratum granulosum), Asterisk = stratum corneum. Scale bar = 10 μm.

FIGURE 6. Section through the skin of an upper limb bud, 6 days *in vitro*. Pulse labelling for 1 h. Marginal zones of the epidermis. Arrow = stratum corneum, E = labelled epiderm cells of basal and intermediate layers. Scale bar = 10 μm.

After 4 days, as on day 3 of development, labelled cells were seen in all epidermal layers, except in the newly formed stratum granulosum. After 5 days *in vitro* it was found that in some regions only the cells of the basal layer were labelled.

After 6 days *in vitro* in almost all skin areas where the epidermis showed a regular order, only cells of the basal layer were labelled (Fig. 5). It was striking that in marginal areas, which result from the removal of the limb bud from the embryo, a thickened epidermis with labelling in all basal and intermediate layers could be seen (Fig. 6). Here the cells seemed to balloon and the entire epidermis structure appeared disturbed.

Pulse labelling for 1 *h and investigation up to* 10 *h after labelling*

As described above, after 3 days *in vitro* labelled cells were present in all epiderm and periderm cell layers after 1 h labelling. During the 10-h follow up period no changes in the labelling pattern of the individual epiderm cell layers could be observed (Figs 3 and 4) indicating that cell migration in a vertical direction from one cellular layer to the other is unlikely during this 10-h period.

DISCUSSION

When comparing the results of our investigations on the labelling of skin cells after application of ^{3}H-thymidine, a relatively complete picture of the cell kinetics of epiderm and periderm cells during skin development and differentiation *in vitro* is obtained.

Labelling with ^{3}H-thymidine for a 24-h period suggests that proliferation of epiderm cells *in vitro* is fastest between days 1 and 2. Application of ^{3}H-thymidine for such a long period causes labelling of all cells that pass through the S-phase during these 24 h. Since with a shorter generation period more cells pass through the S-phase within 24 h than with a longer generation period, the percentage of labelled cells constitutes a rough parameter for the length of the individual cell cycle. Since on day 2 almost all epiderm cells are labelled and the percentage of labelled cells decreases from day 3 onwards, proliferation of epiderm cells is fastest on day 2 *in vitro*.

It is evident that this rapid increase in epiderm cells occurs before the two-layered epidermis becomes multilayered on day 3. The different degree of development of the various epiderm layers from day 4 *in vitro* on might suggest that certain mechanical factors influence their development, since the limb bud cultures lie on filter paper and can grow only in an upward direction against gravity. Hence, certain possibilities of a mechanical influence result which can also be observed macroscopically (Neubert *et al.*, 1974).

On day 6 *in vitro*, after a 24-h ^{3}H-thymidine labelling, only the cells of the basal skin layer are labelled. Therefore it can be concluded that from day 5 to 6 the skin does not grow, since if it grew it would be expected that at least some of the labelled cells in the basal layer would migrate upwards into the intermediate zone. In contradiction to this observation, pulse labelling shows that on day 6 labelling of basal epiderm cells can still be recognized. Long-term labelling with ^{3}H-thymidine for 24 h possibly results in inhibition of cell proliferation, as described by several authors (Painter, Drew & Hughes, 1958; Berry, Oliver & Reiskin, 1966). Without thymidine and with a short-term labelling the skin still grows to a certain extent on day 6. Inhibition of cell proliferation by thymidine on day 2 and 3 is probably very little and does not interfere with these rapidly proliferating cells, while between days 5 and 6, when cell proliferation proceeds only very slowly under the culture conditions it causes an arrest of cell growth.

Pulse labelling with ^{3}H-thymidine for 1 h on day 3 and 4 and partly also on day 5 showed that during the early stage of epidermogenesis each cell layer has its own cell cycle. This may be concluded from the fact that labelled cells *and* mitoses are recognizable in each cell layer. The same independent proliferation behaviour is also true for periderm cells. Cells of the intermediate layer do not change their proliferative behaviour when they change from cuboid to flat epithelial cells. The same applies to cells of the basal layer during transformation from cuboid to cylindrical epithelial cells.

Lack of labelling in the stratum granulosum on days 4, 5 and 6 indicates that these cells no longer proliferate. The transformation from horizontal to vertical proliferation starts with the formation of the stratum granulosum on day 4 and seems to be completed with the appearance of the stratum corneum on day 5. At this stage horizontal growth of each individual epiderm cell layer is replaced by vertical growth starting from the basal layer. These findings support the conclusions that were drawn from investigations on location and orientation of mitosis in the various cell layers during epidermogenesis of mouse embryos *in vivo* (Smart, 1970).

Certain zones of the epidermis, particularly those located at the edges of the explants, did not follow this proliferation pattern, but showed—even in the presence of a recognizable cornified layer—labelling in all layers of the stratum basale and the stratum intermedium. This is possibly due to the preparation of the limb buds during their removal from the donor embryo. The increased proliferation of the zones at the edges of the explant may be attributed to excessive regeneration processes in these wound areas.

The assumption that during the early phase of epidermogenesis, prior to the formation of a stratum granulosum, each newly formed cell layer has its own cell cycle and that the cells grow horizontally, is also supported by the pulse labelling on day 3 and the subsequent observation of the labelling pattern for 10 h. Since the labelling pattern of the stratum basale, stratum intermedium and periderm has not changed 10 h after labelling, it may be concluded that within this period no appreciable cell migration in a vertical direction has taken place. Furthermore, it is of importance that mitoses were observed in all cell layers. It would be unlikely that two non-successive phases—the S-phase and mitosis—take place in one cell layer, whereas the G_1- and G_2-phase proceed in another cell layer. Like the single epiderm layers the periderm grows by itself, i.e. once it has developed it has its own cell cycle. New periderm cells are formed by proliferation in their own layer and not by migration of epiderm cells from beneath.

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Factitious urticaria: Red Dermographism

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SUMMARY

A type of reaction of the skin to superficial trauma is described which differs from ordinary dermographism. This is more easily evoked by rubbing and consists of an erythematous band in which diffuse wealing develops which can be palpated and more easily demonstrated by stretching the skin. Ordinary dermographism is excluded since a scratch as usually employed to demonstrate this produces erythema and not a well-defined raised weal. Forty patients who showed this reaction are described and it was also seen in three out of 100 normal subjects tested.

A type of response of the skin to superficial trauma which does not fit in with described patterns of dermographism has been recognized in the past few years. This is referred to as Red Dermographism (RD).

When testing for dermographism in the usual way the skin is scratched with the nail edge or a blunt pointed instrument. A positive response occurs when a weal develops at the site of the scratch in a few minutes. In an obvious case of ordinary dermographism the weal is 2 mm or more across, is raised and has well-defined margins. There is erythema of the adjacent skin spreading out from the weal for a distance of 5–10 mm.

In patients demonstrating RD the reaction is more easily evoked by rubbing. The area and surrounding skin become erythematous and shortly after this a diffuse weal is apparent in the erythematous skin. This can be palpated and if the erythema is then reduced by stretching the skin the wealing is apparent and may be patchy or involve the whole area. A scratch on the same patient will produce erythema for 5–10 mm from the scratch but no definite central weal and thus ordinary dermographism is excluded.

Many dermatologists will be familiar with patients who explain that weals develop at scratched sites and indeed they may observe red marks with some linearity. Nevertheless when these patients are scratched in the usual way to evoke dermographism, the well-defined, raised, broad weal of ordinary dermographism is not apparent.

Red dermographism is not an uncommon condition and during the last 2 years forty patients have been collected in whom this type of response has been demonstrated. Such a common entity must have been noted many times in the past but no reference to the condition in the last three decades has been discovered. One reason for its lack of recognition is the inexact mode of elicitation and assess-

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ment. Also, as with ordinary dermographism, minor degrees occur which shade into a 'normal' response to scratching or rubbing trauma.

MATERIALS AND METHODS

Patients

Of the forty patients collected over a 2-year period in whom RD had been demonstrated, twenty-six were female and fourteen male. The average age was 41 years and ranged from 13 to 90 with a predominance in the 35 to 55 year age range. The patients had all been routine referrals to out-patient clinics except for nine cases referred specially by dermatologists. They all complained of widespread itching which had been present for an average of 1·4 years with a range from 4 weeks to 5 years. Most patients noticed the relationship to rubbing and the condition was often worse after being in hot surroundings, after hot baths and on sweating after exercise. Many of the patients appeared tense and anxious. Twelve of the patients also had mild clinical eczema of the seborrhoeic eczema pattern, four had chronic urticaria and two psoriasis.

Test trauma

Scratch. This was carried out with either the dermographic instrument (James & Warin, 1969), set to give a pressure of 4900 g/cm^2, or in some cases the edge of the thumb nail. In all cases the upper middle back was the site used.

Rubbing. This was carried out with the back of the middle finger nail stroked over a 20–30 mm length on the upper middle back with a degree of pressure causing the subject slight discomfort. In ten patients the scratching and rubbing were also carried out on the upper arm, thigh and front of the abdomen. The sites were examined 3–5 min after the test trauma although fifteen patients were also observed at intervals from 1–120 min. RD was recorded as present if the rubbed site developed redness and a palpable weal which could be visibly demonstrated by stretching the skin and so reducing the erythema in the area. The weal was usually diffuse over the area rubbed but sometimes was irregular, giving a mottled appearance.

RESULTS

Scratch

None of the forty patients showed the well-defined, raised weal more than 2–3 mm broad, as seen in ordinary dermographism. However, in all cases there was a marked erythema spreading 10–15 mm from the scratched line.

Rubbing

All the patients demonstrated RD over the back and there was a varying degree of itching at the sites. In the ten patients tested, the other sites also demonstrated RD but the reaction was less over the limbs than on the trunk.

Timing

RD starts to develop in 1–2 min and is maximal at 5–6 min. The onset is, therefore, more rapid than with ordinary dermographism. The weals disappear in about 15 min and the red mark persists at the site for about 1 h.

In four patients, after the site of the RD had virtually cleared at 1 h, new rubbings were made across the line of the initial rubbing and repeated in ½ and 1 h. The same degree of RD was noted in these further rubbed sites and therefore a refractory period after the weal was not demonstrated such as has been noted with ordinary dermographism.

In one patient rubbing over the site after 48/80 had been injected produced the usual weal response; however, this observation will need repeating before any conclusion can be made concerning a role of histamine production in the weal. Certainly the use of antihistamines by mouth in these patients appears to reduce the itching and the degree of wealing.

Progress of patients

Only eighteen patients have been followed up for long periods: four cleared completely in an average time of 13 months (range 6–24 months); eleven showed considerable improvement with less or no itching in an average time of 4½ months (range 1–8 months) and three were unchanged at 6, 12 and 18 months respectively.

Normal subjects

100 patients, colleagues, friends and relatives have been subjected to the same scratching and rubbing trauma over the upper back. The patients had such conditions as benign neoplasms, warts, moles, alopecia, etc. There was approximately the same age and sex distribution. Two of the subjects showed an ordinary dermographic weal and were excluded. Three subjects showed RD, two of whom also had some minor scaling and irritation in the scalp. None of these subjects had any itching at the site of the RD.

DISCUSSION

In spite of the use of the dermographic instrument as mentioned above the reaction of the skin to various types of trauma has always been difficult to assess largely because of the lack of standardization of the provoking trauma and also the poor methods of measurement of the erythema and wealing. These difficulties may well account for the fact that RD has not been recorded in the past.

Although RD is more easily evoked by multiple strokes on the same site, in many cases a single stroke is sufficient. Also in cases showing RD a repeated scratch using the dermographic instrument does not evoke a dermographic weal. It therefore seems unlikely that RD is due to more frequent stimulation of the skin at the site in subjects with minor degrees of dermographism.

As RD does not give rise to itching in the normal subjects, there may be a similar position to that seen in ordinary dermographism when in many cases no itching occurs at the site of the weals but in 'Symptomatic Dermographism' there is, of course, considerable itching (Kirby *et al.*, 1971).

Cholinergic dermographism is a rare phenomenon seen in some cases of severe cholinergic urticaria. In these cases the scratch trauma gives rise to an area of erythema studded with tiny weals characteristic of this condition. None of the cases of RD had cholinergic urticaria and RD gives a more diffuse wealing in the erythematous area.

There appears to be an association of RD with seborrhoeic eczema although this was in most cases of a minor degree.

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Further evidence for increased light sensitivity in patients with malignant melanoma

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SUMMARY

Minimal erythema dose (MED), immediate pigment darkening (IPD) and skin typing were performed on 130 patients with malignant melanoma and on seventy-three controls. The tumours were histologically classified according to Clark as superficial spreading melanoma, nodular melanoma, and lentigo maligna melanoma. The tumours of twelve patients were not possible to classify. Patients belonging to skin type IV and patients with lentigo maligna melanoma had a significantly higher mean age. The IPD reaction was significantly higher in skin type II than in skin types III and IV both in patients and in controls. IPD and MED showed no significant correlation to age and sex. MED was significantly lower among patients with malignant melanoma than controls. These data indicate that light sensitivity is of aetiological importance in malignant melanoma.

Epidemiological data have shown a rapid rise in the incidence of malignant melanoma (MM) during the last thirty years (Magnus, 1973; Rhodes, Sober & Fitzpatrick, 1979; Third National Cancer Survey 1969–1971, 1975). Sunlight (UV-B flux) has been considered a major risk factor mainly because of the inverse relationship between geographical latitude and incidence of MM. The highest rates of MM have been reported from countries at lower latitudes with a population consisting mainly of individuals of fair complexion and caucasian origin (Lancaster, 1956; Swerdlow, 1979; Teppo *et al.*, 1978). Black people have a much lower incidence and their lesions are located mainly on the foot (Fleming *et al.*, 1975). The direct importance of sunlight as a risk factor is complex. When MM is compared with basal cell and squamous cell carcinomas in which sunlight without doubt is of significance one finds certain differences. The age distribution differs and the anatomical localization of the lesions is far more complex for MM in relation to most light exposed areas as compared to basal cell and squamous cell carcinomas (Fitzpatrick *et al.*, 1977; Urbach, Davies & Forbes, 1966). The differences in degree of pigmentation and incidence of MM indicate that individual light sensitivity might be of aetiological importance. In an attempt to quantify the individual light sensitivity

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we have measured the minimal erythema dose (MED) (Wennersten & Swanbeck, 1974) and classified skin types (Melski *et al.*, 1977) of 130 MM patients and seventy-three controls.

MATERIALS AND METHODS

Patient material

The material consists of 130 consecutive cases of MM and seventy-three controls. Patients and controls were classified with regard to skin type, and individual light sensitivity was measured with a light testing technique.

Histopathological classification

Depending on the size of the skin biopsy, one to several pieces of the tumour were prepared for histology and stained with haematoxylin and eosin according to routine procedures. In some cases Masson's stain for pigment was used.

The melanomas were classified as proposed by Clark and co-workers (Elder, Ainsworth & Clark, 1979) into the following groups: malignant melanoma, superficial spreading type (SMM); malignant melanoma, lentigo maligna type (LMM); and malignant melanoma, nodular type (NM).

Classification of skin type

The patients normal pre-disease reaction to solar radiation was evaluated. The criteria as stated by Melski *et al.* (1977) are based on the history of their usual reaction to the first hour of full sun exposure in early summer in Sweden. The degree of erythema and pigmentation usually obtained was assessed as follows: Skin type I—always burn, never tan; II—always burn, sometimes tan; III—sometimes burn, always tan; and IV—never burn, always tan.

Light testing

Light testing was performed with an Osram high pressure Xenon arc lamp (XBO 150 W) in a Zeiss Microscope lamp housing with Quartz collector to produce a round, uniformly bright spot, 1·5 cm in diameter, at an exposure distance of 15 cm from the back of the patient to the filter-holder.

The lamp was used with a Schott WG 295 filter, thus giving a solar spectrum-like radiation including both UVB and UVA, with the intensity of the beam at this distance, 28 mW cm^{-2}. The intensity was measured with a Hewlett & Packard radiant flux meter. Irradiations were performed on the untanned mid-back skin and the minimal erythema dose (MED) was established for each patient. The reactions were read after 24 h and the dose was noted for the slightest yet clearly perceptible minimal erythema. Patients and controls were tested for similar time periods.

In order to investigate any possible pathological sensitivity to UVA, the Xenon lamp was furthermore equipped with a combination of a 3 mm glass filter and a KG I heat protective filter, thus excluding wavelengths below 320 nm, and the intensity was then 7·5 mW cm^{-2}, and maximal exposure for occurrence of the immediate pigment darkening (IPD) phenomenon was noted for seventy-seven patients and seventy-two controls.

RESULTS

Among 130 MM patients investigated SSM was documented in 73·8% (96/130), NM in 13·1% (17/130) and LMM in 3·9% (5/130) as shown in Table 1. It was not possible to classify definitely 9·2% (12/130) of the cutaneous melanomas.

Skin types I and II were found in 23·0% of the MM patients. The corresponding figure in controls

TABLE 1. Total number of cases and distribution (in %) among the different tumour and skin types

	Skin type I	%	Skin type II	%	Skin type III	%	Skin type IV	%	Total	%
SSM	2	1·5	24	18·5	59	45·4	11	8·5	96	73·8
NM			4	3·1	12	9·2	1	0·8	17	13·1
LMM					3	2·3	2	1·5	5	3·9
Not classified					9	6·9	3	2·3	12	9·2
Total	2	1·5	28	21·5	83	63·9	17	13·1	130	
Controls			9	12·3	57	78·1	7	9·6	73	

was 12·3% but only skin type II was represented. The differences were statistically significant ($\chi^2 = 3{\cdot}10$, $P<0{\cdot}05$).

The mean age for LMM patients was 67·6 ± 11·7 years which was significantly higher than for SSM ($P<0{\cdot}01$) and NM ($P<0{\cdot}05$) when calculated with t-test. Patients with SSM and NM had a similar mean age ranging from 50·9 to 51·7 years. The highest mean age was also seen for MM patients with skin type IV, 61·5 ± 9·5 years. The higher mean age for tumour occurrence in skin type IV proved to be statistically significant when assessed by analysis of variance (ANOVA), $P<0{\cdot}01$. The significant difference remained even when the patients with LMM were excluded ($P<0{\cdot}05$, Table 2).

In controls age was not found to be correlated to MED or IPD. The squared correlation coefficient found for age/MED was r^2 0·00 and 0·05 for males and females respectively, and corresponding figures for age/IPD were 0·01 and 0·24. Furthermore, sex was not found to be correlated to MED or IPD, when assessed for the largest group of controls, the one with skin type III ($n = 57$). Similarly, when the melanoma patients with SSM and skin type III were considered ($n = 59$) no significant correlation was found between age and MED: r^2 was 0·19 for men and 0·08 for females.

Light testing of MM patients revealed in general a significant lower mean MED when compared to controls ($P<0{\cdot}01$). When the mean MED was calculated for each tumour type no significant differences were found except for LMM patients who had a ($P<0{\cdot}05$) significantly higher MED than the other MM patients and similar to that found in controls. In controls the mean MED increased with skin type as seen in Table 3, i.e. the mean MED was significantly lower for individuals with skin type II compared to those with skin type III ($P<0{\cdot}05$) or skin type IV ($P<0{\cdot}01$). The difference between skin types III and IV represents an increase which was, however, not statistically significant. On the contrary MM patients showed a low mean MED for all skin types. MM patients and controls

TABLE 2. Mean age ± s.d. (years) at diagnosis in relation to tumour and skin type

	Skin type I	Skin type II	Skin type III	Skin type IV	Total mean value
SSM	41·5	51·3	50·6	59·9	51·7 ± 14·1
NM		57·0	48·0	62·0	50·9 ± 17·5
LMM			65·7	70·5	67·6 ± 11·7
Total mean value	41·5 ± 13·5	52·1 ± 16·7	51·2 ± 14·3	61·5 ± 9·5	

TABLE 3. Mean MED value ± s.d. (seconds) in relation to tumour and skin type compared with controls

	Skin type I	Skin type II	Skin type III	Skin type IV	Total mean value
SSM	13·0	11·0	11·8	14·4	11·9 ± 5·1
NM		10·0	12·8	18·0	12·5 ± 5·7
LMM			14·7	21·0	17·2 ± 9·5
Total mean value	13·0 ± 7·0	10·9 ± 3·9	12·1 ± 5·3	15·1 ± 6·4	12·3 ± 5·2
Controls:					
Total mean value		10·7 ± 6·2	15·1 ± 7·7	18·6 ± 4·1	14·8 ± 7·3

with skin types I and II had a similarly low mean MED but the difference between those with skin type III showed that the MM patients had a significantly lower MED ($P<0{\cdot}01$).

The mean IPD threshold dose was calculated for MM patients ($n = 77$) and controls ($n = 72$) for each skin type as seen in Table 4. In controls with skin type II 9·0 J/cm^2 or more was needed to elicit an IPD reaction and several individuals were devoid of any sign of visible IPD phenomenon. For skin type III the mean IPD threshold dose was significantly lower, ($P<0{\cdot}05$), 3·8 ± 2·3 J/cm^2, and still lower for skin type IV, 2·8 ± 1·9 J/cm^2. The MM patients investigated displayed similar mean IPD threshold doses for each skin type but there was no significant difference when patients and controls were compared (Table 4).

TABLE 4. Mean immediate pigment darkening (IPD) threshold doses for different skin types in controls and patients with malignant melanoma (MM)

	Mean IPD threshold doses ± s.d., J/cm^2	
Skin type	Controls	MM patients
II	9·0	9·0
III	3·8 ± 2·3	3·6 ± 5·7
IV	2·8 ± 1·9	3·5 ± 5·5

DISCUSSION

In the present study 130 consecutive cases of malignant melanoma were investigated and the main clinical and histological types of melanoma, i.e. SSM, NM and LMM, were present in a proportion similar to that seen in the majority of previously reported studies of malignant melanoma (Veronesi *et al.*, 1978). Thus, the patients in this study probably constitute a representative sample of MM patients.

Skin types observed revealed a significant over-representation of skin types I and II in MM patients compared to controls indicating that MM seems to be more common in individuals that burn easily and experience a poor delayed tanning capacity. Furthermore, the significantly higher mean age among patients with skin type IV (Table 2) seems to imply that individuals less sensitive to solar radiation and with the best pigmentation properties are better protected and thus develop their tumours at a later stage of life.

MED is thought to be a well established parameter for investigation and assessment of individual light sensitivity but MED is known to be higher before puberty, lower during pregnancy, and seasonal variation may occur (Scott & Molhuysen-v.d. Walle, 1958; Magnus, 1976). Controls and MM patients in the present study were all adults above the age of twenty and none of the females was known to be pregnant. Controls and MM patients were tested on untanned skin and to avoid divergencies due to season no tests were performed during the summer.

Characteristics of the MED and IPD reaction in normal controls were recently investigated and reported elsewhere (Beitner & Wennersten, 1980). As no correlations between these parameters and sex or age were found the control material used in the present study has not been strictly age or sex matched. Similarly, within the MM group no correlations were found between sex and age in relation to MED and IPD when the largest group with skin type III was investigated.

In controls the mean MED for skin type II was significantly lower than for those with skin types III and IV as could be anticipated. Similar differences between skin types were not observed among MM patients who as a group had significantly lower mean MED as compared to controls and also displayed consistently low MED values for all skin types. It therefore seems likely that patients with malignant melanoma may represent a more uniform group with lower mean MED irrespective of skin type. It must be noted, however, that individual MED and IPD threshold values varied within each skin type among both controls and patients.

The mean age for tumour occurrence increased with skin type. Patients with skin types I and II developed their tumours at a significantly lower age than those with skin type IV. The significant difference remained even when the patients with lentigo maligna melanoma (LMM) were excluded. LMM occurs at a significantly higher mean age than other malignant melanoma as confirmed in the present study. LMM has a fairly slow growth phase and has the best prognosis. LMM patients in this study had skin type III or IV and also normal MED values compared to the controls which is further evidence of the clinical and histological characteristics seen in this special type of malignant melanoma.

In skin types I and II most patients and controls were lacking the IPD reaction under the experimental conditions used, with a maximum dose up to 15 J/cm^2. The biological significance of the IPD reaction is uncertain but the phenomenon may provide further protection against solar radiation. As skin type I or II was nearly twice as common in MM patients compared to controls the lack of IPD reaction perhaps may facilitate the development of cutaneous malignancies, in this instance MM.

Fair complexion is said to be over-represented in patients with malignant melanoma and this was confirmed in the present study with an increased proportion of individuals easily burned and with deficient or poor tanning capacity when both delayed pigmentation and the immediate pigment darkening phenomenon were considered (Lancaster & Nelson, 1957; Gellin, Kopf & Garfinkel, 1969). Furthermore, MM patients had lower MED values without a significant increase with skin type. These findings might provide further indirect evidence for the observation that individuals with increased light sensitivity, i.e. skin types I and II, are more prone to develop malignant melanoma. Other factors seem also to be involved as MM patients as a group (except LMM patients) have lower MED values than controls, a difference seen also for those with skin types III and IV. Delineation of populations at risk for malignant melanoma must include those with skin types I and II but individual habits of degree and manner of solar exposure seem to be of equal importance and remain to be investigated. Such studies are now in progress.

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Eye protection in psoralen photochemotherapy

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SUMMARY

The optical performance of some commercially-available tinted lenses is discussed. There is a wide variation in the UV absorbing power of sunglasses although adequate protection against solar UV-A can be achieved with some, but not all, polarizing material. The darkness of a tint is not an indication of its likely performance within the ultraviolet bands and it is possible that lenses could be produced that lacked any significant tint yet absorbed strongly throughout the UV region.

Oral psoralen photochemotherapy (PUVA) is now widely used for the treatment of psoriasis and other skin diseases. One of the potential drawbacks of long-term therapy associated with this treatment is the possibility of producing cataracts, a fear that has arisen from animal experiments (Cloud, Hakim & Griffin, 1960). Eye damage has been produced in guinea-pigs who were administered oral 8-methoxypsoralen and whose eyes were irradiated with monochromatic radiation from 300 to 390 nm in steps of 10 nm (Freeman & Troll, (1969). There was evidence of ocular injury at 72 h post-irradiation at all wavelengths less than 380 nm, with maximum photosensitivity occurring between 320 and 340 nm. Eye protection is provided during patient treatment by using goggles which are opaque to ultraviolet radiation (UVR), e.g. Portia Actinotherapy Goggles, Solport Bros. Ltd.

Solar UV-A irradiance on cloudless days has been found to be as high as 5 $\mathrm{mWcm^{-2}}$ (Parrish *et al*, 1978); a value which is comparable with that encountered in treatment units. Since most of the UV-A that enters the pupil is absorbed by the crystalline lens of the eye (Boettner & Wolter, 1962), and since free 8-methoxypsoralen can be detected in human lenses for at least 72 h following oral ingestion (Lerman, Megaw & Willis, 1980), it is desirable that patients should wear suitable eye protection for the remainder of the day to reduce the ingress of UV through the pupil to insignificant levels. The cornea of the eye absorbs UV below about 300 nm but transmits increasingly above this wavelength. There is therefore a need for UV absorbing filters to be worn. The council of perfection is for the patient to wear an appliance in which the lenses do not transmit wavelengths less than 400 nm and which incorporate side-shields (e.g. Blak-Ray Contrast Control Spectacles, Model No. UVC-303, Ultra-Violet Products Inc.). However, in spite of the technical merits of this and possibly other

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similar appliances, it cannot compete in cosmetic appeal with commercially available sun-spectacles. Some of these may in fact provide sufficient protection and what is more important, the patient is likely actually to wear them.

PERFORMANCE OF TINTED LENSES

A simple method of assessing UV-A transmission through sunglasses was described by Wennersten (1978) using a UV-A meter and the lamps used in a PUVA treatment unit. This technique was critically examined by Diffey & Miller (1980) who concluded that in general the results obtained by the method could be extrapolated to the performance of the sunglasses in sunlight. The purpose of the present communication is to expand the work of Wennersten by presenting transmission spectra of various commercially available tinted lenses and discussing their performance.

Tinted lenses can be divided into two main groups. The first group comprises pure industrial filters which protect against harmful radiations on either side of the visible spectrum (both infrared and ultraviolet radiations) and, in addition, they reduce transmission within the visible spectrum. Sunglasses and tinted prescription lenses that have evolved from the original industrial glass materials are within this group. The second group are those where the principal consideration is the reduction of visible radiation, i.e. glare, and may or may not have protective properties as well. In addition, within this group are sunglasses which are worn principally for their cosmetic effect.

When protection against UV-A radiation is required, as in patients undergoing PUVA therapy, the first group, i.e. industrial filters and their derivatives, are unsuitable. Their function is to absorb well in the short wave UV (UV-B and UV-C) in order to prevent various forms of photo-ophthalmia. This form of kerato-conjunctivitis is caused by radiations within the wavelength range from around 250 nm to 330 nm. The condition, when it occurs in industry, is called by such terms as 'arc eye' and 'welder's flash'. It also occurs at high altitude where UVR is less attenuated by earth's atmosphere and may be reflected upwards into the eye unprotected by the brows. Although the denser shades of welding filters may provide protection against UV-A, as well as the shorter UV wavelengths, they are so dark within the visible spectrum as to be impractical for non-industrial use. So industrial protective filters are unsuitable for patients who need protection against UV-A.

An initial survey of the transmission properties of sunglasses supplied by Boots showed a wide range of UV absorption performance. The results indicated that, in general, sunglasses with non-polarizing lenses had only moderate to poor absorption in the UV—some exhibiting more than 20% transmission at 300 nm. The present survey concentrates on those materials which perform well as UV-A absorbers. All transmission spectra presented here were obtained by placing the lens in a recording spectrophotometer.

Polarizing sunglasses use a stretched layer of cellulose acetate containing iodine crystals sandwiched between two support layers. Cellulose butyrate is commonly used for the support layers. Additives to absorb ultraviolet radiation are usually included in the support layers. Not all polarizing lenses examined performed equally well as demonstrated by the transmission spectra illustrated in Fig. 1. One of those available which used a thermosetting resin, allyldiglycocarbonate (CR-39), on either side of the polarizing layers, was a poor absorber of ultraviolet radiation, having a cut off point little different from that of normal crown glass used for clear prescription lenses. Crown glass decreases in transmission at wavelengths below 360 nm and is virtually opaque to wavelengths less than 300 nm. The other brands of polarizing sunglasses were tested and two of these would appear to obtain their lens materials from the same source. The largest producer is the Polaroid Corporation whose material is made in America, exported in sheet form around the world and made up into sunglass lenses in various Polaroid factories. (The Polaroid factory in Scotland serves Europe.)

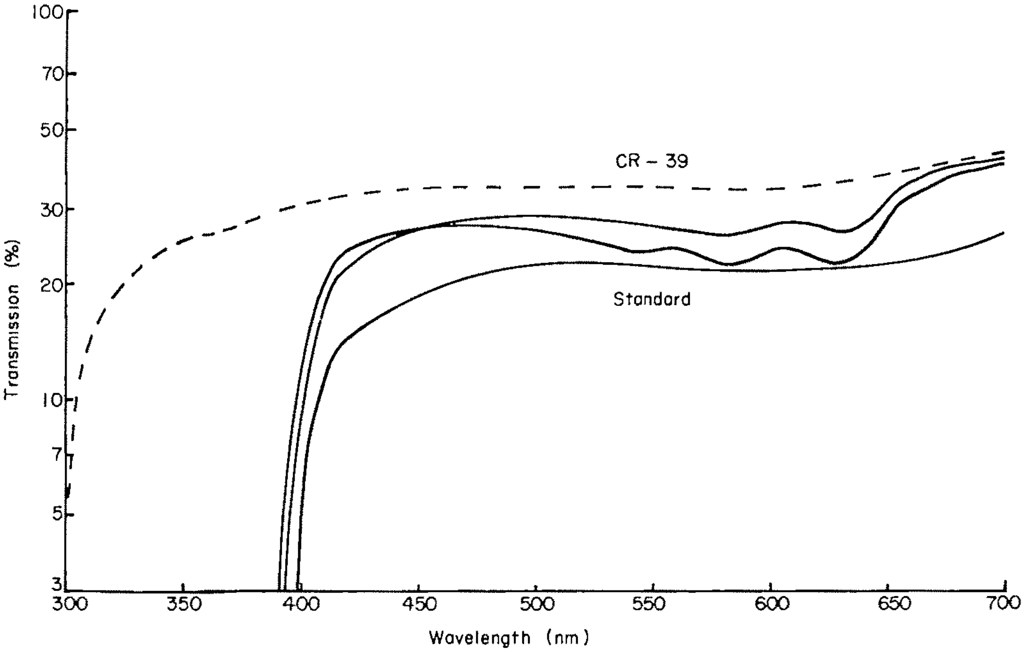

FIGURE 1. Polarizing sunglasses vary widely in their ability to absorb wavelengths less than 390 nm. One CR-39 (allyl resin) plastics sunglass transmits to below 300 nm. The standard Polaroid sunglass has a transmission of 24% within the visible spectrum but negligible transmission in the UV-A region. The transmission curves of two other polarizing brand name sunglasses are shown, which appear to use the same source of filter material and also exhibit negligible transmission in the UV-A.

The polarizing sunglasses made by the Polaroid Corporation all have a high performance as UV-A absorbers. Three different densities of tint are used in currently available sunglasses, the densest having a 16% transmission of light, the normal having 25% transmission and the lightest having a 39% transmission. There is little difference in their UV absorptive performance (Fig. 2). In order to conform with the dictates of fashion, Polaroid introduced coloured lenses to supplement their earlier range of three neutral tints. These yinted lenses are in fact the Polaroid 39% transmission polarizing material, with additional coloured dyes in the support layers. Because of the neutral tint of the polarizing layer these tints were 'muddy' compared with those of the lenses of their competitors. Polaroid, whose lens sheet material production was geared to the basic three layer process, therefore removed the polarization treatment of the interlayer. These non-polarizing lenses still retain the UV absorption characteristics of their polarising lenses and have been given the name UV-96 to indicate that they absorb 96% of incident UV energy. Unfortunately, Polaroid also market acrylic and CR-39 lenses which do not perform so well as either their polarizing lenses or their UV-96 materials. The Polaroid UV-96 lenses transmit up to 76% of visible light and even more is transmitted by the clearer parts of some of the lenses with a graduated tint that becomes progressively clearer in the lower zones of the lens.

Another lens of even higher transmission in the visible spectrum is in spectacles incorporating an amber lens marketed by Bausch and Lomb in their Ray-Ban Kalichrome glass. The visible transmission is claimed to be 75% while the UV-A absorption is better than any of the other lenses examined. Several photochromic glasses are now produced which alter in density according to the local

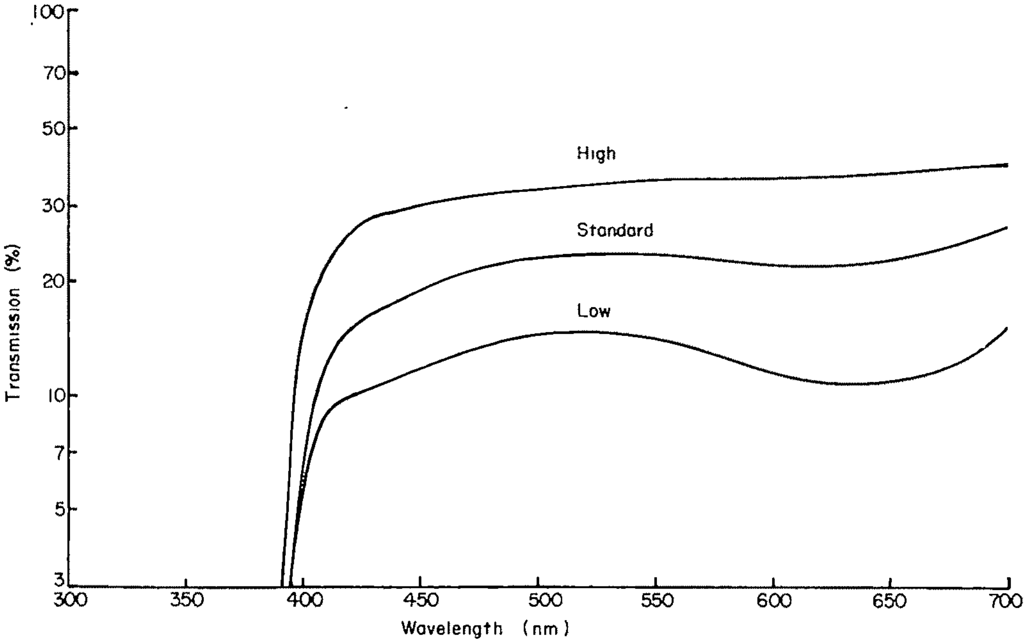

FIGURE 2. The density of the filter within the visible spectrum does not provide a guide to the performance within the ultraviolet region. The high, the standard and the low transmission versions of the Polaroid polarizing sunglass all cut off at around 390 nm.

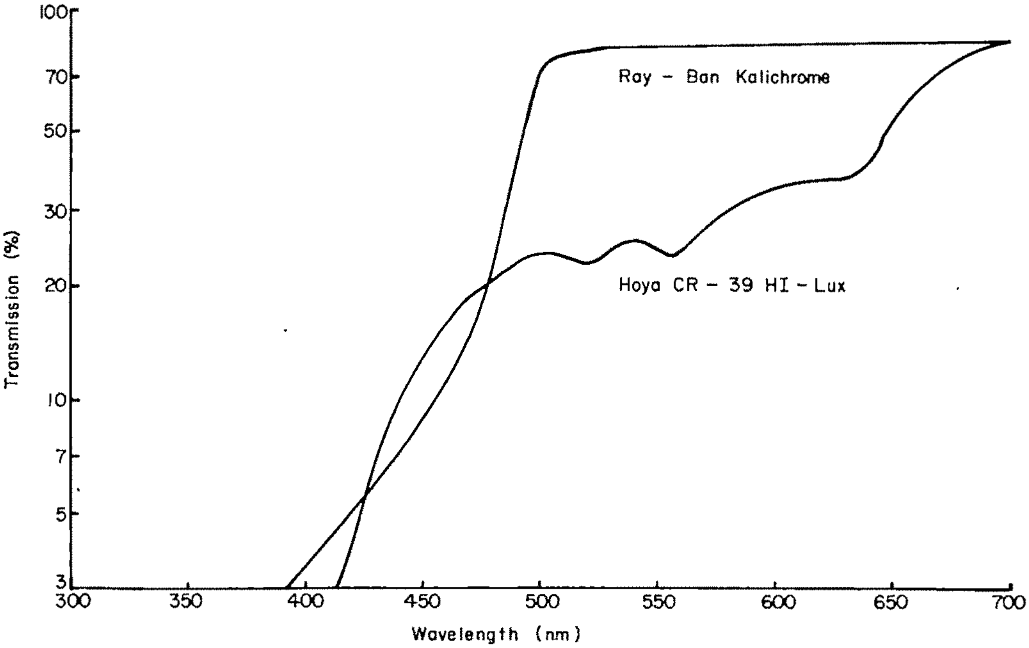

FIGURE 3. Two filters with good ultraviolet absorption. The Bausch and Lomb Ray-Ban Kalichrome uses an amber lens and the Japanese Hoya Company obtain a brown coloured CR-39 (allyl resin) lens by dyeing the surface.

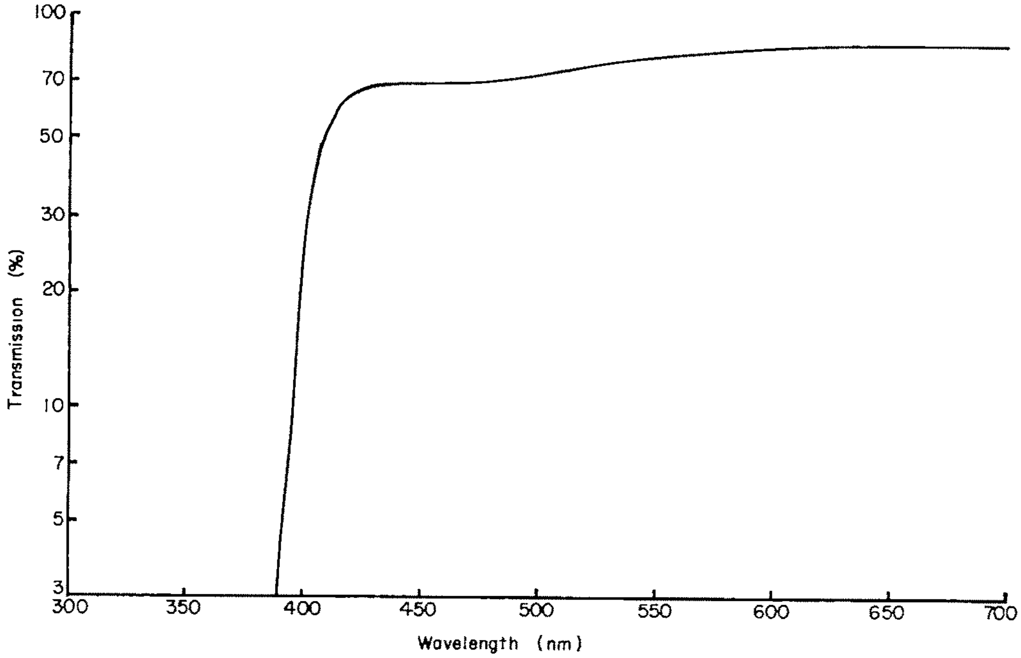

FIGURE 4. The ultraviolet absorber is in the two 'support layers' of Polaroid sunspectacles of the traditional sandwich construction. The clear portion of a non-polarizing amber and clear graduated tint sunglass lens has a negligible transmission below 390 nm.

ambient light level. Unfortunately, they have all been designed to match up in performance to industrial protective glasses of similar density and so absorb strongly below 360 nm, i.e. they transmit UV-A with wavelengths between 360 and 400 nm even when they are fully darkened, and so may be unsuitable for use in psoralen therapy.

The ametrope needs to have a spectacle lens correction in addition to protection from UV-A. The Polaroid 'clip-ons' are all made from either their various polarising materials or their less dense UV-96 material. Prescription lenses could be made in Bausch and Lomb Kalichrome glass, although they are not normally available. An alternative solution could be the plastics amber CR-39 lens produced by the Japanese Hoya Corporation. This brown lens has a tint produced by surface dying. The integrated visible transmission is about 25% which makes it as dark as most sunglasses (Fig. 3)

Now that the hazard of the possible cataractogenic effects of UV-A on patients being treated with oral psoralen is appreciated it is possible that a lens which absorbs as much as the best lenses covered in this survey but without a significant tint could be produced both in unpowered form for people who do not wear glasses for distance vision and also as a coating for those who require a powered lens to prescription (Fig. 4). In the latter case it would seem a simpler solution to use a plastics lens and then to treat the surface of the lens so as to give it the necessary absorption properties in the UV-A. This would eliminate the aversion response in which the pupil dilates in response to the decreased visible light levels associated with tinted lenses.

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Cyclic GMP metabolism in psoriasis: increased activity of soluble epidermal cyclic GMP phosphodiesterase and its modulation by calcium

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SUMMARY

Soluble cyclic GMP-phosphodiesterase was measured in normal and psoriatic epidermis. The specific activity of the enzyme was increased almost four-fold in involved compared with normal epidermis, and two- to three-fold in involved compared with uninvolved epidermis. The enzyme activity from all three sources was inhibited by 40–50% by ethylene glycol tetraacetic acid (EGTA). These results indicate that in addition to the reported enhanced capacity of psoriatic epidermis to generate cGMP, it has an increased ability to hydrolyse this nucleotide, although to a lesser degree than the augmentation found in soluble guanylate activity from psoriatic epidermis. These observations are compatible with the elevated steady-state levels of this nucleotide observed in the involved epidermis of psoriasis.

Elevated cellular cyclic 3′,5′guanosine monophosphate (cGMP) concentrations have been demonstrated in the involved areas of psoriatic epidermis (Voorhees *et al.*, 1973; Marcelo *et al.*, 1979). We have recently reported a greatly enhanced soluble epidermal guanylate cyclase activity in lesional epidermis (Cantieri, Graff & Goldberg, 1980) as well as a sensitivity of this enzyme activity to stimulation by the two polyunsaturated fatty acids, arachidonic acid and 12-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE), known to accumulate in the lesions (Hammarstrom *et al.*, 1975). Although these findings suggest that the elevated steady-state levels of this nucleotide observed in lesional epidermis are attributable to an enhanced biosynthesis of cGMP, they do not preclude a role for its altered hydrolysis by cyclic GMP-phosphodiesterase (cGMP-PDE). A previous report comparing this enzyme activity in uninvolved and involved psoriatic epidermis showed a modest elevation in the

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activity of the enzyme from involved epidermis (Iizuka *et al.*, 1978). As an initial step in delineating the role of cGMP hydrolysis in the altered metabolism of this nucleotide in psoriasis, we have measured the activity of soluble epidermal cGMP-PDE in normal and psoriatic epidermis and have examined its regulation by the potentially important physiological modulator, calcium.

MATERIALS AND METHODS

Preparation of soluble cGMP-phosphodiesterase

Human skin slices were obtained without anaesthesia from five normal volunteers and four untreated psoriatic patients. Informed consent was obtained from all participants. Both uninvolved and involved areas of skin were sampled from psoriatic patients; the uninvolved specimen was taken from an area of skin at least 5 cm from a well-developed plaque. A Castroviejo keratome set to a tissue depth of 0·1 to 0·2 mm for normal and uninvolved epidermis and 0·4 mm for involved epidermis produced slices 3 cm wide by 8 cm long, which were immediately frozen in liquid nitrogen. Histological examination revealed that the samples were predominantly epidermis with little dermis, but the degree of dermal contamination was variable. The frozen samples were pulverized by mortar and pestle in liquid nitrogen and homogenized with a Brinkman Polytron in 50 mM tris-HCl, pH 7·5, containing 2 mM DTT. The homogenates were centrifuged for 60 min at 105,000 *g* to separate the soluble and particulate cellular fractions. The soluble fraction was removed and assayed within 30 min.

cGMP-phosphodiesterase assay

Phosphodiesterase activity was determined according to a method recently described by Walseth & Johnson (1979). The assay was conducted at 30 °C in a total reaction mixture of 100 μl. Final concentrations of components in the assay were 1 μM cyclic [^{32}P]-GMP (250,000 c.p.m./nmol), 2·5 mM $MgCl_2$, 50 mM tris-HCl, pH 8·0. Ten microlitres of soluble extract (30–250 ng protein) was added to initiate the reaction. When desired, a final concentration of 1 mM EGTA was added to the reaction mixture prior to the addition of the enzyme. Upon termination of this reaction by heating to 90 °C for 90 s, $^{32}P_i$ was liberated from the [^{32}P]-GMP formed by the addition of 56 ng (3 μl) of snake venom 5′nucleotidase (Sigma) and a subsequent incubation at 37 °C for 30 min. This reaction was stopped by the addition of 1·0 ml 0·1 M H_3PO_4 containing 25 mg of acid-washed Norit A (Fisher Scientific). The unhydrolysed cyclic [^{32}P]-GMP was absorbed quantitatively to the charcoal, which was removed by centrifugation. The $^{32}P_i$ in the supernatant fraction was determined by Cherenkov counting. Under these conditions the reaction was linear for at least 30 min and reaction blanks conducted with boiled enzyme or no enzyme were no more than 0·2% of the total c.p.m. present in the assay mixture. The protein concentration of the extract was determined by the method of Bradford (1976). All determinations were conducted in triplicate. Specific activities are expressed as pmol/min/mg protein ± s.e. mean. Significance levels were established by a paired, two-sided Student t-test.

RESULTS

cGMP-phosphodiesterase activity

The phosphodiesterase (PDE) activity promoting cGMP hydrolysis was examined in soluble extracts from the three types of tissue. Employing 1 μM cCMP as substrate, the enzyme activity from each source was linear for at least 30 min, but the specific activity of the enzyme from the involved epidermis of the psoriatic was found to be considerably greater (3–4-fold) than that from normal and from uninvolved epidermis. Table 1 is a summary of the cGMP-PDE specific activities from the three types of epidermis. The enzyme activity from involved tissue is almost four times greater than that found in

TABLE 1. Cyclic GMP phosphodiesterase specific activities

Normal	Uninvolved (U)	Involved (I)
19·50	14·50	54·50
14·98	9·31	63·60
6·69	24·38	21·20
6·10	21·60	38·70
Mean ± s.e. mean 11·6 ± 2·5	17·5 ± 3·4	44·5 ± 9·3
	(U/N 1·51 NS)	(I/N 3·85 $P<0{\cdot}01$)
		(I/U 2·55 $P<0{\cdot}05$)

Units—pmol/min/mg protein.
NS—not significant.

normal epidermis. The activity from uninvolved epidermis of psoriatics, however, is intermediate, but variable. The cGMP-PDE specific activity from involved epidermis was, however, significantly greater than that detectable in uninvolved or normal tissue.

Inhibition by EGTA

Since phosphodiesterases from various sources have been shown to be activated by or require calmodulin plus calcium (Goldberg & Haddox, 1977; Kakiuchi & Yamazaki, 1970; Means & Dedman, 1980), the effect of ethylene glycol tetraacetic acid (EGTA) on epidermal cGMP-PDE was examined. These experiments were conducted not only to establish whether the cGMP-PDE in human epidermis may be regulated by Ca^{2+}: calmodulin, but also to determine whether a different species of cGMP-PDE, distinguishable by its sensitivity to Ca^{2+}, may be present in psoriatic epidermis where total hydrolytic activity is enhanced. EGTA (1 mM) caused a 40–50% inhibition of cGMP-PDE from each of the three types of tissue (Fig. 1).

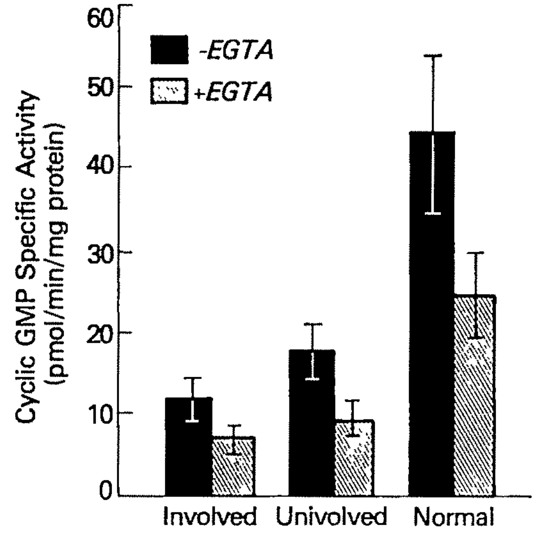

FIGURE 1. Effect of EGTA (1 mM) on soluble cyclic GMP phosphodiesterase specific activities. EGTA was added to the reaction mixture and the reactions were carried out as described in the methods section. Solid bars represent basal specific activities without EGTA; cross-hatched bars represent activities in the presence of EGTA. The tops of the bars are means ± s.e.mean.

DISCUSSION

Iizuka *et al.* (1978) have published the only previous study of cGMP-PDE activities in involved and uninvolved psoriatic epidermis. Measuring the activities in crude homogenates from microdissected epidermis, they found a 40% increase in the activity of the low K_m enzyme from involved epidermis, but no difference when comparing activities from undissected keratomed slices. The only previous report of soluble cyclic nucleotide phosphodiesterase activities in psoriatic epidermis by Voorhees *et al.* (1974) showed a 3–4-fold increase in soluble cAMP-PDE activity in involved compared with uninvolved epidermis. Our findings of a 2–3-fold enhanced cGMP-PDE activity in involved compared with uninvolved epidermis, and a 4-fold increased activity in involved compared with normal epidermis, are more consistent with the data from the latter report. As in our previous report of guanylate cyclase activity, uninvolved psoriatic epidermis manifested an alteration in PDE activity to an intermediate and lesser degree than that seen in the enzyme activity from involved epidermis.

In this examination of the activity of the enzyme hydrolysing cGMP in psoriatic epidermis, it seemed appropriate to limit the study to only the low K_m PDE activity, since substrate concentrations previously utilized in the examination of the high K_m enzyme (Iizuka *et al.*, 1978) were in a less physiological range (75–100 μM) than the 1 μM cGMP substrate concentration chosen for the present studies. Evidence from the chromatographic separation of distinct enzyme species suggests that the high K_m enzyme activity probably represents a species of PDE with cAMP as the preferred substrate and cGMP as a structurally related, low-affinity substrate.

EGTA, a specific chelator of Ca^{2+}, was nonspecific with respect to the selectivity of its inhibitory effect on the three types of PDE. Whereas Iizuka *et al.* (1978) reported only a 10–20% nonselective inhibition by EGTA, we have found a much greater decrease (40–50%) in specific activity caused by this agent (Fig. 1) and, therefore, a greater role for Ca^{2+}:calmodulin in the regulation of epidermal cGMP-PDE activity and cGMP turnover than previously thought. It has been demonstrated, moreover, that in many tissues cGMP-PDE activity is more sensitive than cAMP-PDE to modulation by calcium plus calmodulin at low substrate concentrations (Goldberg & Haddox, 1977; Kakiuchi *et al.*, 1973). Choice of nucleotide substrate may thus account for the different degrees of PDE inhibition by EGTA reported by the two groups.

Psoriatic epidermis, then, appears to possess an increased ability to both synthesize and hydrolyze cGMP, but there remains a greater net increase in synthetic than hydrolytic capacity, which may explain the elevated steady-state levels of this nucleotide observed in psoriatic epidermis (Voorhees *et al.*, 1973; Marcelo *et al.*, 1979) and postulated to be associated with enhanced rates of cell proliferation (Hadden *et al.*, 1972; Goldberg *et al.*, 1974; Seifert & Rudland, 1974).

Another conclusion that can be drawn is that the increased activity to generate and to hydrolyze cGMP in psoriatic epidermis supports a greatly accelerated turnover rate of this nucleotide. The possible utility of cGMP turnover is obscure at this time but it is conceivable that it may relate to an 'energy-providing' process, such as that in which cGMP-PDEs have been suggested to function (Kakiuchi & Yamazaki, 1970), especially those modulated by Ca^{2+}:calmodulin.

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Epidemiology of congenital pigmented naevi: I. Incidence rates and relative frequencies

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SUMMARY

Pigmented naevi (PN) were clinically diagnosed in 1083 newborn infants from a series of 531,831 consecutive livebirths examined in fifty-nine hospitals in South America. Isolated PN (IPN) were seen in 989 (0·2%) non-malformed infants, while PN associated with other congenital anomalies (APN) were observed in ninety-four (1·0%) malformed babies. Incidence rates calculated from the fifty-nine hospitals were very variable due to under-reporting, which might have been as high as 80%. The PN were classified by their location, number, size, texture, and colour. Observed locations were compared with random expected distributions based on the surface proportions of the newborn. IPN were preferentially found on the chest and abdomen, with a low concentration on the head and upper limbs. No examples were observed on soles or palms. IPN were single in 90% of cases. Size was small (1–9 mm) in 29%, medium (10–40 mm) in 63%, and large (over 40 mm) in 8% of cases. Abnormal texture, (rugose, raised, and/or hairy) was found in 7–11% of IPN. Colour was brown in 68%, black in 21%, and of other colours in 11%. The large IPN tended to be multiple in number and abnormal in texture. Small IPN tended to be black. IPN on the chest and abdomen were more frequently small, non-black, and of normal texture; those found on the back tended to be black and abnormal in texture; black IPN were also frequent on the upper limbs; large IPN frequently affected the lower limbs. The APN did not differ from the IPN in their number, size, texture, or colour. APN were less common than expected on the back and upper limbs. An association was observed between the PN and the following four congenital anomalies: ear deformities, preauricular appendages, angiomas, and other skin anomalies. The differences in distribution of PN in the newborn infant and that in the adult that have been previously published suggest that most of the PN found in the adult are not congenital.

The congenital pigmented naevus is a common condition of the newborn infant, either as an isolated finding in an otherwise normal baby, or as part of a more generalized syndrome. The information available in the literature concerning the incidence and relative frequencies of the different morpho-

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logical types of congenital pigmented naevi, as well as their relationship with the pigmented lesions of the adult, is limited to a few investigations (Pratt, 1953; Pack & Davis, 1956; Walton, Jacobs & Cox, 1976).

The purpose of this paper is to describe the relative frequencies of congenital pigmented naevi as related to their clinical morphological characteristics, and their associations with congenital developmental anomalies, as observed in an unselected sample of consecutive newborn infants.

MATERIALS AND METHODS

The material presented here includes 1083 newborn infants with pigmented naevi (PN), clinically diagnosed by the attending paediatrician within the first 3 days of life. They were all the newborn babies with PN ascertained through the physical examination of 531,831 livebirths, performed in fifty-nine hospitals participating in the Latin American Collaborative Study of Congenital Malformations: E.C.L.A.M.C. (Castilla *et al.*, 1973) within the 1967–1976 period. Therefore, the sample includes fifty-nine series of consecutive births, corresponding to the fifty-nine involved hospitals, in twenty-five cities of seven South American countries: Argentina, Brazil, Chile, Ecuador, Peru, Uruguay, and Venezuela.

All circumscribed, hyperpigmented lesions of the skin, excluding mongolian spots, were considered as PN. The number of lesions, size, location, colour, texture, elevation above the skin level, and presence of abnormal hairs, were described according to instructions given in a procedures manual.

This material of 1083 PN cases, out of 531,831 total livebirths, included 989 cases of isolated PN among 521,836 non-malformed newborn infants, and ninety-four cases of associated PN among 9995 malformed babies.

RESULTS

1. *Incidence rates*

An overall incidence rate for isolated pigmented naevi (P.N) cases cannot be derived from the data of 989 observed cases among 521,836 non-malformed newborn babies (0·2%) due to the variations in the frequencies reported from the fifty-nine different hospitals (χ^2: 1,844·70; df: 58; $P<0{\cdot}001$). The highest incidence rate was in a hospital with 255 isolated PN cases detected in 25,892 livebirths (1·0%). Since the variations observed among hospitals may reasonably be interpreted as due to reporting differences, rather than to real variation in the population, it could be estimated that the frequency of isolated PN cases in the present sample should have been at least 1%. Therefore, the rate of under-reporting in the whole fifty-nine hospital sample was 80%, as derived from an expected frequency of 1·0% and an observed one of 0·2%.

2. *Morphological characteristics*

The distribution frequencies for five external morphological characteristics of the isolated and associated PN are shown in Table 1.

Location. The exact location on the body surface for each PN was reported in all but three of the 989 isolated PN cases, and in all but one of the ninety-four associated PN cases. Cases were grouped into six areas of the body surface, and those cases having more than one affected area (sixty-four isolated, and six associated) were excluded from further analysis. Therefore, the effective totals were 922 cases with isolated PN, and eighty-seven cases with associated PN.

Within the isolated PN group, 29·1% had the PN located on the lower limbs, 26·4% on the chest and abdomen (anterior trunk, excluding the genital area), 13·8% on the craniofacial area (head, face, and neck), 12·0% on the back (posterior trunk, excluding the sacro-perineal area), 11·0% on the upper limbs, and 7·8% on the sacro-perineo-genital area (including buttocks).

Since the larger the area the greater the probability for a PN to be located on it just by chance, the analysis of location was based on the randomly expected frequency distribution derived from the

TABLE 1. Morphological characteristics of pigmented naevi as observed in non-malformed (isolated) and malformed (associated) infants

	Isolated naevi 989 cases		Associated naevi 94 cases		
Characteristics	*n*	%	*n*	%	*P**
Affected area					
Craniofacial	127	13·8	20	23·0	+
Chest/abdomen	243	26·4	18	20·7	—
Back	111	12·0	3	3·4	+
Sacro-genital	72	7·8	10	11·5	—
Upper limbs	101	11·0	8	9·2	—
Lower limbs	268	29·1	28	32·2	—
Total	922	100·0	87	100·0	
	(67)†		(7)		
Number					
Single	899	90·9	86	91·5	—
Multiple	90	9·1	8	8·5	
Total	989	100·0	94	100·0	
Size (mm)					
Small (01–09)	268	28·8	31	37·4	—
Medium (10–40)	590	63·3	49	59·0	
Large (≥41)	74	7.9	3	3.6	
Total	932	100·0	83	100·0	
	(57)		(11)		
Texture					
Normal	554	88·6	47	85·5	—
Abnormal	71	11·4	8	14·5	
Total	625	100·0	55	100·0	
	(364)		(39)		
Colour					
Brown	422	68·3	38	74·5	—
Black	129	20·9	6	11·8	
Other	67	10·8	7	13·7	
Total	618	100·0	51	100·0	
	(371)		(43)		

* Significance of differences between isolated and associated naevus groups. Contingency chi-square test. —($P > 0·05$): not significant, +($P < 0·05$): significant.

† Number of cases with unspecified data in parentheses.

proportion of the total body surface corresponding to each of the six defined areas in the neonatal age group (Lund & Browder, 1944). When this comparison was done, a preferential location of PN on the chest and abdomen was evident, with twice as many cases as expected. On the contrary, less than expected were observed on the craniofacial and upper limb areas, while the three other regions (back, sacro-genital, lower limbs) had the expected proportion. Not a single case was reported on the palms or soles (Table 6).

Number. The information concerning the presence of single or multiple PN in the same infant was available for all cases. The exact number of PN was not stated in thirteen isolated cases, reported as having 'several' PN. Due to coding limitations, nine cases were pooled into the class having eight or more PN. Within the isolated PN group, 90·9% of cases had a single PN (Tables 1 and 2).

Size. The size of each PN was taken as that of its greatest diameter, in millimetres, and the size of the largest one was considered for analysis when there were more than one. The size was specified in 932 isolated, and in eighty-three associated PN cases. Small PN (<10 mm) were seen in 28·8% of the isolated PN patients, medium size (10–40 mm) in 63·3%, and large (>40 mm) in 7·9% (Tables 1 and 2).

Texture. The texture of the PN was considered to be either normal, when similar to the surrounding skin, or abnormal, when rugose, raised, and/or hairy. These descriptions were specifically stated for only 63·2% (625/989) of the isolated, and for 58·5% (55/94) of the associated PN cases. Since a tendency to omit the description of negative (normal) rather than of positive (abnormal) findings may be suspected, the observed frequencies of the PN with abnormal textures (11·4% of the isolated cases) must be considered as maximal estimates. On the assumption that all unspecified cases had a normal texture, 7·1% of isolated cases would have PN with abnormal textures. The real frequency of abnormal texture PN must lie in between those two estimates: i.e. 7–11%, approximately. The seventy-one

TABLE 2. Number of newborn infants with pigmented naevi, classified by size and number of naevi

Size (mm)	Number									
	1	2	3	5	6	7	8≥	M*	Total	CU %‡
01–04	64	4	1					3	72	7·7
05–09	181	10	2				1	2	196	28·8
10–14	182	10		1					193	49·5
15–19	98	9	1	1				1	110	61·3
20–29	151	6						1	158	78·2
30–40	124	3	1					1	129	92·1
41–80	36	7	1			1	2	1	48	97·2
99≥	15	1	3		1		4	2	26	100·0
Total	851 (48)†	50 (4)	9 (1)	2	1	1	7 (2)	11 (2)	932 (57)	

* Number of cases with more than one naevus, number unspecified.
† Number of cases with unspecified size in parentheses.
‡ Cumulative percentage.

cases with isolated PN of abnormal texture included: twenty hairy, seventeen rugose, thirteen raised, and twenty-one with more than one of these three elements (Table 1).

Colour. The colour of the PN was not specified in a large proportion of cases, usually being referred to as hyperpigmented naevi. Colour was stated in 618 isolated, and fifty-one associated cases. Of the former group, 68·3% were brown, 20·9% black, and 10·8% had other colours, mainly blue and gray (Table 1).

Correlations. The following morphological correlations were identified. The large PN (>40 mm) tended to be multiple (31·1%) and abnormal in texture (36·7%). The small PN (<10 mm) were more frequently black in colour (27·2%) than the larger PN (Table 3).

The PN located on the chest and abdomen tended to be small (34·9%), normal in texture (95·4%), and non-black in colour (87·8%). The PN on the back had the highest frequency of abnormal texture (18·9%), while the highest frequency of black PN was found on the back and on the upper limbs (30·6% each). The PN on the lower limbs were mostly larger than 10 mm (78·3%) (Table 4).

TABLE 3. Frequency of multiple lesions, abnormal texture, and black colour, among small, medium, and large isolated pigmented naevi

		Multiple naevi			Abnormal texture			Black colour		
Size (mm)	*n*	*n*	%	*P**	*n*	%	*P**	*n*	%	*P**
Small (1–9)	268	23	8·6		23	11·9		50	27·2	
		(0)†		—	(74)		—	(84)		++
Medium (10–40)	590	35	5·9		26	7·1		61	16·8	
		(0)		++	(225)		++	(226)		—
Large (41≥)	74	23	31·1		18	36·7		14	29·2	
		(0)			(25)			(26)		
Total	932	81	8·7		67	11·0		125	21·0	
		(0)			(324)			(336)		

* Significance of differences between small and medium, and medium and large naevi. Contingency chi-square test. —($P>0\cdot05$): not significant, ++($P<0\cdot01$): significant.

† Number of cases with unspecified data in parentheses.

3. *Associated PN*

The incidence rate of PN observed in malformed newborn infants (94/9995: 1·0%) was significantly higher than that observed among the non-malformed babies (989/521,836: 0·2%) (χ_1^2: 313·98; $P<0\cdot001$). Nevertheless, the frequency of PN reported from newborn infants with a single malformation (75/8509: 1·0%) did not differ from that of the multiply malformed babies with two or more malformations (19/1486: 1·3%) (χ_1^2: 0·70; $P<0\cdot70$).

The PN found in the malformed newborns did not differ from the isolated PN in their morphological characteristics of number, size, texture, or colour. As in the isolated PN, the associated PN were found on the lower limbs with a less than expected frequency. The craniofacial area was more frequently affected in the associated (23·0%) than in the isolated (13·8%) PN group. Since such frequencies were similar to the expected value (21·0%) in the former, it can be said that the associated

TABLE 4. Frequency of small size, abnormal texture, and black colour among isolated pigmented naevi within each affected area

		Small size			Abnormal texture			Black colour		
Affected area	*n*	*n*	%	*P**	*n*	%	*P**	*n*	%	*P**
Craniofacial	127	38 (5)†	31·1	—	11 (54)	15·1	—	13 (59)	19·1	—
Chest/abdomen	243	81 (11)	34·9	+	7 (92)	4·6	++	19 (87)	12·2	++
Back	111	33 (8)	32·0	—	14 (37)	18·9	+	22 (39)	30·6	+
Sacro-genital	72	18 (7)	27·7	—	6 (29)	14·0	—	11 (28)	25·0	—
Upper limbs	101	31 (4)	32·0	—	9 (38)	14·3	—	19 (39)	30·6	+
Lower limbs	268	55 (15)	21·7	++	14 (96)	8·1	—	34 (101)	20·4	—
Total	922	256 (50)	29·4		61 (346)	10·6		118 (353)	20·7	

* Significance of differences between each affected area and the rest of the body surface. Contingency chi-square test. $-(P>0{\cdot}05)$: not significant, $+(P<0{\cdot}05)$ and $++$ $(P<0{\cdot}01)$: significant.

† Number of cases with unspecified data in parentheses.

PN did not spare the craniofacial area, as the isolated PN did. The opposite was found for the lesions on the back, which were less frequent in the associated (3·4%) than in the isolated (12·0%) PN group, the latter being similar to the expected 13·0% (Table 1).

There were forty-four different types of congenital malformation found in association with the PN, twenty-five of them observed in more than one case. Nevertheless, a significant association could be proven for only four categories: ear deformities, preauricular appendages, angiomas, and other skin anomalies excluding PN and angiomas, mainly represented by supernumerary nipples and skin tags (Table 5).

4. *Severe PN*

A group of severe PN could be defined by selecting those cases with PN at least 10 cm in diameter, with eight or more PN, or with PN of abnormal texture (rugose, raised, and/or hairy). Within the isolated PN group, ninety-four cases fitted into this definition: sixty-three because of abnormal texture, eighteen because of giant size, three due to a great number of lesions, four due to size and texture, two due to number and size, two due to number and texture, and two due to number, size and texture. Within the associated PN group there were nine severe PN cases, one because of giant size, and the rest because of abnormal texture. The proportion of severe PN cases, assuming all unspecified observations were not severe, was approximately 10% for both the isolated (94/989) and the associated (9/94) groups.

It may be reasonably assumed that the rate of under-reporting did not affect the large, more conspicuous, PN. Therefore, since PN larger than 10 cm were observed in twenty-six newborn infants, and this size class may be considered as giant for this age group, it may be estimated that the incidence rate for giant PN is 1/20,455 (26/531,831) livebirths, and also that 23·1% (6/26) of those

TABLE 5. Preferential association of pigmented naevus and congenital malformations in the same patient: observed and expected number of cases within a sample of 531,831 live births

Congenital malformation		Associated with pigmented naevus			
Type	Observed number	Observed number	Expected number	Observed/ expected	P*
Skin anomalies†	400	15	0·82	18·40	+++
Preauricular appendages	1302	16	2·65	6·04	++
Ear deformities	351	10	0·71	14·02	+
Angiomas	504	10	1·03	9·73	+
Talipes talo-valgus	299	6	0·61	9·81	—
Polydactyly	851	6	1·73	3·46	—
Talipes equino-varus	798	5	1·63	3·07	—

* Significance of differences between expected and observed numbers. Goodness of fit by chi-square test. —($P>0·05$): not significant, +($P<0·05$), ++ ($P<0·01$), and +++($P<0·001$): significant.

† Other than pigmented naevus and angiomas.

giant PN are already hairy at birth. The bathing-trunk distribution was reported in only one case in the whole sample.

DISCUSSION

The interest in defining incidence rates and relative frequencies for pigmented naevi (PN) in the newborn infant is related to the need for baseline data from the beginning of life onwards, in order to understand the natural history of PN. Few studies of PN in the newborn period have been published, and all of them involved small samples. Only three investigations were specifically directed towards PN (Pratt, 1953; Pack & Davis, 1956; Walton *et al.*, 1976), whereas three other studies, which were basically concerned with congenital anomalies, provided data on PN in the newborn (Marden, Smith & McDonald, 1964; Hay, 1971; Harris *et al.*, 1975). Even though small studies have the advantage of allowing more uniform, homogeneous, and detailed observation, they have the drawback of low statistical significance. None of the dermatological studies mentioned above included much more than a thousand births. Therefore, even though the observed incidence rates seem to differ among them: 58/1096 = 5·3% (Pratt, 1953), 41/1058 = 3·9% (Walton *et al.*, 1976), 5/200 = 2·5% (Pack & Davis, 1956), such differences are not significant and may be explained by sampling variation (χ^2_2: 4·28; $P>0·05$). On the other hand, studies involving a large number of observations have the opposite advantages and disadvantages. Lower incidence rate figures are obtained due to under-reporting of the PN: 21/4412 = 0·5% (Marden *et al.*, 1964), 88/21,142 = 0·4% (Harris *et al.*, 1975), 16/57,909 = 0·03% (Hay, 1971). The observed incidence rate of 1083/531,831 = 0·2% in the material presented here falls into this second category of surveys. Under-reporting of PN probably involved only the less conspicuous mild PN, while the observed frequencies for the severe types of PN, mainly those with a size of 10 cm or greater, are more likely to be reliable.

The relevance of the study of site is related mainly to the risk of malignant change by correlation with the distribution patterns for malignant melanomas (Cullen, 1962), as well as to a knowledge of the PN life history (Lund & Stobbe, 1949). The observed proportions of PN on each area of the body are of little value when the long term natural history of PN is being considered because of the substantial changes in the proportions of the body occurring from birth into adult life. Therefore, the affected areas of the skin must be analyzed as observed/expected ratios, instead of as crude observed percentages. The analysis of site was referred to the time of diagnosis, at birth, rather than to the time when the PN might actually be laid down, in prenatal life, because no data are available concerning surface proportional distributions in the fetus, and, furthermore, the developmental age of appearance of congenital PN is also unknown, even though there are indications that it might be as early as the second trimester of intrauterine life (Harrison & Okun, 1960).

In order to compare the data presented here with that of other published studies, the chest, abdomen, back, and sacro-perineo-genital areas were grouped together into a single area: the trunk (Table 6). In the newborn infant there was a preferential location of PN on the trunk, with lower than expected numbers on the head and upper limbs, and the expected numbers on the lower limbs.

TABLE 6. Observed and randomly expected distributions of the isolated pigmented naevi by body surface areas in newborn infants and in adults

	Newborn data				Adult data						
	Present study *n*: 922 cases				Stegmaier & Becker, 1960 *n*: 1079 naevi				Lewis & Johnson, 1968 *n*: 2575 naevi		
			O/E				O/E			O/E	
Affected area	E (%)*	O (%)†	Ratio	*P*‡	E (%)*	O (%)†	Ratio	*P*‡	O (%)†	Ratio	*P*‡
Craniofacial	21	14	0·7	++	9	20	2·2	++	11	1·2	+
Trunk	32	46	1·4	++	32	33	1·0	—	34	1·1	—
Chest/abdomen	13	26	2·0	++	—	—	—		—	—	
Back	13	12	0·9	—	—	—	—		—	—	
Sacro-genital	6	8	1·3	—	—	—	—		—	—	
Upper limbs	19	11	0·6	++	19	32	1·7	++	22	1·2	++
Lower limbs	28	29	1·0	—	40	15	0·4	++	33	0·8	++

* Expected random proportion of naevi.

† Observed proportion of naevi.

‡ Significance of differences between observed and expected proportions. Goodness of fit by chi-square test. —($P>0{\cdot}05$): not significant, +($P<0{\cdot}05$) and ++ ($P<0{\cdot}01$): significant.

When the adult data published by Stegmaier & Becker (1960) and by Lewis & Johnson (1968) were re-analyzed considering the adult body surface proportions (Lund & Browder, 1944), both studies shared a preferential location of PN on the head and on the upper limbs, with less than expected on the lower limbs, and as expected on the trunk. The high percentage of PN on the lower extremities, as well as the low proportion affecting the head in adult patients, mentioned in the literature (Lewis & Johnson, 1968), simply reflect the body surface proportions, with the head and neck representing only 9·0% of the adult body surface, and the lower limbs amounting to as much as 40·0% of it. The changing pattern on PN distribution frequency from birth to adult life cannot be entirely explained by changes in the proportions of the body. While the relative size of the head diminishes from 21·0% to 9·0%, its proportion of PN increases, and the reverse may be observed for the lower limbs. Neither the trunk nor the upper limbs undergo any modification in their relative sizes from birth to adult life, even though a substantial reduction in the proportion of PN on the trunk and an increase of those on the upper limbs take place between these two age periods.

The lack of cases with PN located on the palms or soles among 1083 affected newborn infants is a highly significant negative observation since the expected figure is close to 30%, as based on Cullen's (1962) data from young adult males of Latin American extraction.

The observed differences in site of PN between the newborn and adult patients suggest that a large proportion of the adult PN are not congenital, as well as that some pathogenic differences may exist. The concept that congenital PN tend to be larger in size and more frequently premalignant in nature was based on retrospective data from adult patients (Mark *et al.*, 1973) and, therefore, subject to information bias, as was pointed out by Walton *et al.* (1976). The clinical and histological investigation of neonatal PN by Walton *et al.* (1976) showed that one third of the congenital pigmented lesions were histologically melanocytic naevi, and that 4/11 of these measured 15 mm or more.

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HLA antigens in patients with scabies

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SUMMARY

Sixty patients with scabies were typed for thirty-three antigens of the HLA-A, -B and -C series. A significantly increased frequency was found for HLA-A11 (28·3%), compared to healthy controls (10·4%). This deviation was only found in those of the patients without signs of atopic disease.

The cell-membrane molecules which are determined by the closely linked genes in the HLA chromosomal complex may be divided into two different classes (Thorsby, 1979):

(a) The *HLA-ABC* molecules, which are determined by allelic genes at the A, B and C loci, are present on probably all nucleated cells and are highly polymorphic.

(b) The *HLA-D/DR* molecules which have a more restricted tissue distribution and are present mainly on B lymphocytes and monocytes/macrophages.

Typing for these antigens has become a tool of steadily increasing interest. Patients with certain diseases have an increased frequency of particular HLA antigens compared to healthy individuals (Dausset & Svejgaard, 1977). This is also true for some dermatological diseases. The strongest appear to be the association between D/DR3 and dermatitis herpetiformis (Solheim *et al.*, 1977), but discoid lupus erythematosus (Stenszky, Nagy & Szerze, 1975), psoriasis (Williams *et al.*, 1976), vitiligo (Retornaz *et al.*, 1976) and lichen planus (Lowe, Cudworth & Woodrow, 1976; Halevy *et al.*, 1979) have been found to be associated with certain HLA antigens. The reasons for these associations are unknown, but probably involve HLA gene control of T cell immune responses (Thorsby, 1978). Immunological mechanisms are also involved in patients with scabies (Mellanby, 1944; Falk, 1980; Falk & Bolle, 1980a,b).

In view of these observations we looked for an association between scabies and any of the HLA-ABC antigens.

MATERIALS AND METHODS

Individuals studied

The present study was carried out on random unrelated patients with clinically manifest scabies. The diagnoses were in all cases confirmed by the presence of one or more living mites. All patients came from the northern part of Norway, but no Lapps were included.

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Sixty patients (thirty-five women and twenty-five men) aged from 2 to 62 years (average 27·3 years) were studied. Sixteen of them were atopic patients, defined as patients with either current atopic disease or a past history of such illness, including atopic dermatitis, allergic rhinitis or extrinsic asthma.

Healthy controls consisted of 1468 Norwegians from different parts of the country: blood donors, members of the staff, parents of patients with uraemia, etc.

HLA-ABC typing

HLA-ABC typing was performed using peripheral blood mononuclear cells procured by Lymphoprep (Nyco, Oslo, Norway) flotation and a microcytotoxicity test (Kissmeyer-Nielsen & Thorsby, 1970). A highly selected battery of fifty-four antisera was used to detect ten antigens of the A series, nineteen of the B series and four of the C series. The HLA D/DR antigens could not be typed for in the present material.

Statistical methods

Any deviations in antigen frequencies were evaluated using χ^2 statistics (without Yates' correction) or Fisher's exact test, depending on the number expected in each entry. If necessary P values were corrected for numbers of antigens investigated (thirty-three). The relative risk (RR) (how many times more frequently the disease is found in individuals carrying a given antigen compared to those lacking it) was evaluated using Woolf's formula (Dausset & Svejgaard, 1977).

TABLE 1. Frequency of HLA antigens in sixty scabies patients and in 1468 healthy controls

HLA locus A	Scabies No.	Scabies %	Controls (%)	HLA locus B	Scabies No.	Scabies %	Controls (%)	HLA locus C	Scabies No.	Scabies %	Controls (%)
A1	17	28·3	29·4	B5	11	18·3	8·8†	Cw1	3	5·0	6·3
A2	31	51·7	56·1	B7	10	16·7	30·2†	Cw2	5	8·3	10·4
A3	20	33·3	30·3	B8	15	25·0	24·9	Cw3	13	21·7	36·2
A9	8	13·3	19·2	B12	14	23·3	29·1	Cw4	2	6·3	19·3
A10	0	0	8·4	B13	0	0	2·2				
				B14	0	0	2·8				
A11	17	28·3	10·4*	B15	7	11·7	19·9				
				B16	0	0	2·8				
				B17	8	13·3	6·2				
A28	4	6·7	7·9	B18	3	5·0	4·7				
A29	3	5·0	6·3	Bw21	2	3·3	1·7				
Aw32	2	3·3	6·1	Bw22	2	3·3	3·4				
Aw19 (30+31)	2	3·3	2·0	B27	7	11·7	11·0				
				Bw35	11	18·3	14·2				
				B37	1	1·7	2·1				
				B40	8	13·3	20·4				
				Bw45	0	0	0·9				
				Bw47	1	1·7	0·8				
				Bw53	0	0	0·2				

* $P<0{\cdot}01$ (after correction).

† $P>0{\cdot}05$ (after correction).

RESULTS

After correction for number of antigens tested for, we found a significant deviation for HLA-A11, which was increased to 28·3% (17/60) in the patients with scabies, compared to 10·4% in 1468 healthy controls ($P<0·01$) (Table 1). The frequency of B5 was also increased, that of B7 decreased, whilst no patients were found to carry A10, but none of these deviations reached statistical significance after correction.

In the non-atopic patients with scabies the occurrence of A11 was even more prominent (36·4%), whereas the atopic patients had the same distribution of antigens (6·3%) as in the normal healthy Norwegian population (Table 2). There was a highly significant difference between atopics and non-atopics with regard to the occurrence of A11 ($P<0·01$). The relative risk of scabies among A11 positive individuals was found to be 3·4 and this further increased to 4·9 for those who were non-atopic.

No correlation between A11 and age, sex or duration of the disease was found.

TABLE 2. HLA-A11 among sixty scabies patients with and without atopic disease, and in 1468 healthy controls

		No.	%	RR
Scabies patients	Total	17/60	28·3	3·4
	Non-atopics	16/44	36·4	4·9
	Atopics	1/16	6·3	—
Healthy controls		153/1468	10·4	—

DISCUSSION

The increased frequency of HLA-A11 in the present series was found only among the non-atopic patients with scabies, whereas the atopic patients had the same distribution of antigens as in the normal healthy Norwegian population. From recent studies it is evident that Lapps have a reduced frequency of HLA-A11—6·82% versus 10·4% (Thorsby, Bratlie & Teisberg, 1971)—but there were no Lapps among our patients. Otherwise, there are no other known geographical or racial variations in HLA antigen frequencies within Norway.

There is at present no evidence from published series of an association between atopy and any particular HLA antigen (Dausset & Svejgaard, 1977; Schultz Larsen, Grunnet & Vase, 1980). The rather complex association found between HLA-A11 and scabies is rather surprising. The most likely interpretation for this association would be that there is an HLA-linked gene increasing susceptibility to scabies and that it is a linkage disequilibrium with A11. In individuals without atopy this gene may operate to increase susceptibility. However, where a genetic predisposition to atopy is present, for some unknown reason the above gene may not be effective and, therefore, indirectly no association with A11 becomes manifest.

As these investigations are, as far as we know, the first where HLA typing has been carried out on patients with scabies, our findings need to be confirmed in other populations.

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HLA-DR3 in dermatitis herpetiformis

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SUMMARY

Twenty-one patients with dermatitis herpetiformis were typed for HLA-ABC and -DR determinants. The incidence of HLA-A1, -B8 and -DR3 antigens was found to be significantly higher (P: $=10^{-3}$, $<10^{-6}$ and $<10^{-6}$, respectively) among patients with dermatitis herpetiformis than among the normal population. HLA-DR3 was found in 85·7% of patients, HLA-B8 in 66·7% and HLA-A1 in 61·9% only. These results indicate that HLA-DR3 is the antigen primarily associated in dermatitis herpetiformis and the latter antigens (HLA-A1 and -B8) are present in increased incidence, probably due to the known linkage disequilibrium of these antigens with HLA-DR3.

A statistically significant association between dermatitis herpetiformis (DH) and the histocompatibility antigen HLA-B8 is well confirmed (Katz *et al.*, 1972; White *et al.*, 1973; Gebhard *et al.*, 1974; Seah *et al.*, 1976; Reunala *et al.*, 1976). Recently, another HLA factor—the determinant HLA-Dw3—has been shown to occur as frequently (Solheim *et al.*, 1976, Keuning *et al.*, 1976) or more frequently (Thomsen *et al.*, 1976) than HLA-B8 in DH. The present study was performed in order to examine the incidence of HLA-ABC and -DR determinants in DH.

PATIENTS AND METHODS

Patients

Twenty-one unrelated patients with DH were studied, fifteen males and six females. They were aged from 11 to 75 years (mean age 43·2±4·3 (s.e.mean) years). In each patient the diagnosis was based on the typical clinical criteria, the prompt improvement of symptoms after sulphone treatment and the characteristic histopathological findings, including subepidermal cleft formation and neutrophil leukocyte accumulation in the tips of the dermal papillae or in adjacent vesicular areas. In all patients granular deposits of IgA were demonstrated in the tips of the dermal papillae of perilesional as well as of clinically uninvolved buttock skin specimens (Katz & Strober, 1978). None of the patients had clinical symptoms of gluten-sensitive enteropathy.

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HLA-typing

HLA-ABC typing was performed using the NIH standard microlymphocytotoxicity assay (Brand *et al.*, 1970). The HLA-DR antigens (HLA-DR 1–5·7) were determined by the two colour fluorescence method, utilizing sera of the 7th International Histocompatibility Workshop (at least three antisera per specificity) (van Rood, Van Leeuwen & Ploem, 1976).

Statistics

Statistical analysis was performed using the χ^2-test with Yates' correction. The relative risk (RR) was calculated according to Woolf (1955).

TABLE 1. HLA-A1, -B8 and -DR 1–5·7 frequencies in dermatitis herpetiformis patients and healthy controls

HLA-	DH		Healthy controls		Statistical analysis		
	Positive	%	Positive	%	χ_2	P	RR
A1	13/21	61·9	820/3000	27·3	10·81	10^{-3}	4·3
B8	14/21	66·7	543/3000	18·1	29·56	$<10^{-6}$	9·1
DR1	3/21	14·3	32/160	20·0	0·11	0·74	0·7
DR2	8/21	38·1	35/160	21·9	1·88	0·17	2·2
DR3	18/21	85·7	32/160	20·0	36·88	$<10^{-6}$	24·0
DR4	3/21	14·3	42/160	26·3	0·85	0·36	0·5
DR5	1/21	4·8	37/160	23·1	2·75	0·10	0·2
DR7	3/21	14·3	45/160	28·1	1·18	0·28	0·4

RESULTS

The typing results in patients with DH and in control individuals are listed in Table 1. The incidence of HLA-A1,-B8 and -DR3 antigens was significantly higher (P: $=10^{-3}$, $<10^{-6}$ and $<10^{-6}$, respectively) among patients with DH than among the unrelated normal healthy volunteers. HLA-A1 was found in 61·9% and HLA-B8 in 66·7% of patients with DH, whereas these antigens were present in only 27·3% and 18·1%, respectively, of 3000 normal persons (relative risks: 4·3 for HLA-A1 and 9·1 for HLA-B8.) The incidence of HLA-DR3 was 85·7% in patients and 20% in 160 control individuals, the relative risk being 24. HLA-B8 was never detected in an HLA-DR3 negative patient; HLA-A1 was determined only in two HLA-B8 negative patients, one of whom was positive for HLA-DR3. The frequencies of HLA-DR 1,2,4,5,7 determinants and of other HLA-ABC factors did not differ significantly between DH patients and controls.

DISCUSSION

The present study indicates that the HLA-DR3 determinant is the antigen primarily associated with DH. HLA-B8 and -A1 are also present in increased incidence in DH, probably due to the known linkage disequilibrium of these antigens with HLA-DR3. These data confirm the results of Thomsen *et al.* (1976) who showed that HLA-Dw3 (formerly LD-8a), a determinant defined by cellular techniques and strongly correlated with the serologically detectable antigen HLA- DR3, is the most frequent HLA-factor in DH. In gluten sensitive enteropathy (GSE), HLA-B8 and -Dw3 are also found in increased frequency and, as in DH, the HLA-Dw3 determinant is the antigen primarily associated (Keuning *et al.*, 1976; Solheim *et al.*, 1976; Thomsen *et al.*, 1976). A large percentage of patients with DH display the morphological changes (flattened villi) of GSE, although in most cases the intestinal disease is clinically inapparent (Marks, Shuster & Watson, 1966; Katz & Strober, 1978). The highly significant increase in the frequency of HLA-B8 and -Dw3 in both disorders has been regarded as evidence for a genetic link between DH and GSE (Katz *et al.*, 1972; Solheim *et al.*, 1976). It has been postulated that the underlying pathogenic factor in both diseases may be a gluten hypersensitivity, which could be genetically determined by genes at a closely linked locus (Solheim *et al.*, 1976; Katz & Strober, 1978).

Additional factors must be involved, however, since the intensity of the intestinal symptoms is different in the two disorders and GSE is not necessarily associated with DH (Karlsson, Dahl & Marks, 1978). It is interesting to note that Thomsen *et al.* (1976) found a significantly increased frequency (34·8%) of HLA-Dw7 (formally LD-12a) in twenty-three patients suffering from both DH and coeliac disease (= GSE) besides the increased incidence of HLA-B8 and -Dw3. In contrast only 13·3% of fifteen DH patients without coeliac disease were positive for HLA-Dw7. This is similar to the frequency of this determinant (8·2%) in the control individuals (Thomsen *et al.* 1976). In the present study, the incidence of HLA-DR7 was lower in patients with DH than in the controls (Table 1). The patients had no clinical evidence for GSE but intestinal biopsies were not performed.

Recently De Marchi *et al.* (1979) and Betuel *et al.* (1980) reported on a significantly increased incidence of both HLA-DR3 and -DR7 in patients with adult coeliac disease, though differences exist in the relative frequencies of DR7 in the several groups of patients investigated, which might be due to ethnic differences. In contrast to Thomsen *et al.* (1976) none of five patients of Betuel *et al.* (1980) who exhibited features of DH was positive for HLA-DR7. Further studies will be necessary to clarify the incidence and significance of HLA-DR7 in DH with and without GSE.

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Pharmacology and Treatment

A clinical evaluation of the effects of an aromatic retinoid (Tigason), combination of retinoid and PUVA, and PUVA alone in severe psoriasis

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SUMMARY

Eighty ambulatory adult patients with severe psoriasis were included in our 14-week study to compare the clinical effects of treatment with an aromatic retinoid (Tigason), combination treatment with aromatic retinoid plus PUVA (RePUVA), and PUVA alone. RePUVA was given by two different modifications: either 4 weeks of pretreatment with retinoid followed by 6 weeks of PUVA treatment, or 10 weeks of retinoid treatment with concomitant PUVA treatment during the last 6 weeks. The latter modification of RePUVA proved to be significantly more effective than the other treatments ($P<0{\cdot}01$) as regards complete remission. With respect to the number of patients with good or excellent improvement (75–100% improvement) there was no marked difference between RePUVA and PUVA treatments. Retinoid alone proved to be the least effective, showing, however, a good to excellent result in 65% of patients. Besides the increase in efficacy, another advantage achieved by RePUVA was a highly significant ($P<0{\cdot}001$) reduction of total UVA doses to about one third as compared to PUVA therapy.

Oral 8-methoxypsoralen photochemotherapy (PUVA) is a generally accepted method for treating severe psoriasis resistant to other therapy (Parrish *et al.*, 1974; Wolff *et al.*, 1976; Melski *et al.*, 1977). PUVA treatment is well-tolerated, rarely causing such acute side-effects as phototoxicity, severe pruritus or nausea. Many patients, however, need rather high UVA doses which by cumulation may exceed even 1000 J/cm^2. In such cases there are increased risks of long-term effects like cutaneous ageing, cataracts and carcinogenicity. Recent reports (Stern *et al.*, 1979; Hofmann, Plewig & Braun-Falco, 1979) have stressed the possibility of neoplastic changes in certain risk-groups. It is therefore desirable to develop modifications which would allow a decrease in the total UVA dose without loss in efficacy.

Various retinoids—derivatives of vitamin A—have been reported to possess moderate to good

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antipsoriatic activity (Lassus, 1980). During recent years an aromatic retinoid (Tigason), has been & found to be effective even in severe cases like erythrodermic and pustular forms of psoriasis (Goerz & Orfanos, 1978; Lassus, 1980). Previous studies of combined treatment with the aromatic retinoid and PUVA, so-called RePUVA therapy, have been promising allowing a marked reduction in the total UVA doses (Orfanos *et al.*, 1978; Fritsch *et al.*, 1978; Heidbreder & Christophers, 1979).

The aim of the present study was to compare the efficacy of and tolerance to treatment with the aromatic retinoid, PUVA and RePUVA in cases of severe psoriasis.

MATERIALS AND METHODS

Eighty ambulatory adult patients with severe psoriasis regarded as acceptable for PUVA therapy were included in the study (Table 1). The patients were randomly allocated into four different treatment groups, twenty patients in each. The design of the study is presented in Fig. 1.

Tigason doses used in groups I, II and III are shown in Table 2. The initial dose was 50–60 mg/day in all three groups during the first 2 weeks. In groups I and III the doses were usually reduced later on during the study, in some cases to 20–30 mg/day, depending on the clinical response and possible side-effects.

PUVA therapy was given according to standard methods. The initial UVA dose was about the same in all cases (1–1·5 J/cm^2) and increased at weekly intervals. The maximum single dose used was 12 J/cm^2. PUVA was initially given 3 times per week; in some cases this fell to 1–2 times per week by the end of the study, depending on the treatment response.

Control examinations were carried out every second week during the 10 weeks of active treatment

TABLE 1. Characteristics of the patients

	No.	Males/Females		Mean age (years) ± s.e.mean	Mean weight (kg) ± s.e.mean	Mean duration of disease (years) ± s.e.mean
Group I	(20)	8	12	43·5 ± 3·1	67·5 ± 2·0	21·6 ± 7·1
Group II	(20)	12	8	41·5 ± 2·9	71·2 ± 2·7	13·3 ± 1·8
Group III	(20)	11	9	44·2 ± 2·9	67·5 ± 2·7	12·1 ± 3·1
Group IV	(20)	8	12	38·9 ± 3·0	68·1 ± 3·8	13·3 ± 2·8

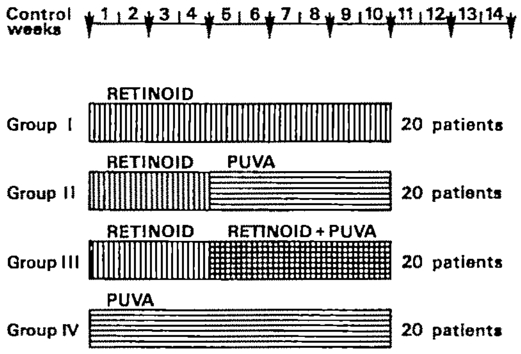

FIGURE 1. Treatment schedules and control visits.

TABLE 2. Mean doses of Tigason used in groups I, II, III. Mean doses (mg) ± s.e.mean during 2-week periods. Dose spread in brackets

	Weeks 0–2	No.	Weeks 2–4	No.	Weeks 4–6	No.	Weeks 6–8	No.	Weeks 8–10	No.
Group I	57·0 ± 1·0 (50–60)	20	47·5 ± 1·6 (40–60)	20	42·1 ± 1·6 (30–60)	19	39·5 ± 1·6 (30–60)	19	36·1 ± 1·4 (30–50)	18
Group II	57·5 ± 1·0 (50–60)	20	49·0 ± 2·0 (40–60)	20						
Group III	57·0 ± 1·0 (50–60)	20	45·5 ± 1·8 (30–60)	20	36.0 ± 1·3 (30–60)	20	33·7 ± 1·4 (30–50)	19	32·1 ± 1·4 (20–50)	19

and thereafter during the 4 subsequent weeks in order to detect any rebound phenomena. Before the treatment started and at each visit the severity of the lesions was evaluated on the basis of erythema, desquamation and infiltration, which were graded from 0–3. The extent of lesions was expressed as approximate percentage of involved skin. Evaluations were made separately on face, scalp and neck, trunk, upper extremities and lower extremities. In order to evaluate the overall severity of the disease, the psoriasis area and severity index (PASI) was determined as described by Fredriksson & Pettersson (1978). The improvement during the treatment was recorded as the amount of reduction in PASI scores using the following grading system: excellent (100%), good (>75%), moderate (>50%), slight (>25%) and poor (<25%).

At each visit possible side-effects were recorded and serum transaminases and alkaline phosphatase were determined.

RESULTS

Before the treatment there was no significant difference in the mean PASI scores between various groups. The overall results of the treatment are shown in Table 3. At the end of active treatment sixty-seven (84%) of the eighty patients showed either an excellent (complete remission) or a good

TABLE 3. Overall results of treatment. The figures are number of patients

Control week	4th				10th				14th			
Group	I	II	III	IV	I	II	III	IV	I	II	III	IV
Improvement:												
Excellent 100% (complete remission)	0	0	0	0	3	5	13	4	2	5	12	5
Good >75%	3	3	3	0	10	12	5	15	9	10	4	11
Moderate >50%	9	7	8	12	5	1	0	1	3	1	0	3
Slight >25%	6	8	7	6	0	1	0	0	3	1	0	0
Poor <25%	2	2	2	2	0	1	1	0	1	2	3	1
Total	20	20	20	20	18*†	20	19†	20	18	19‡	19	20

* One patient interrupted because of severe paronychia

† Two patients interrupted because of flare-up of the disease

‡ One patient did not return for last control visit

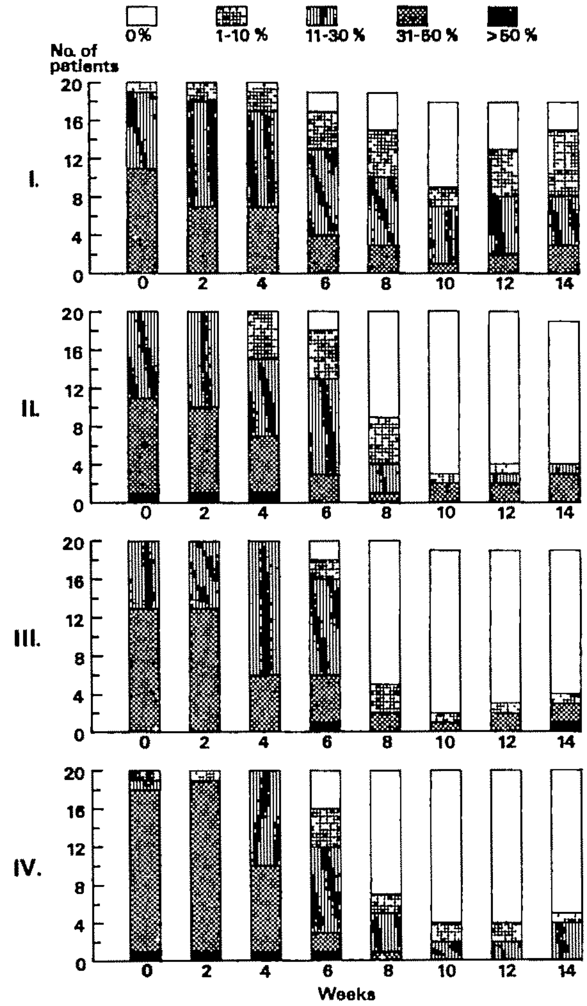

FIGURE 2. Extent of the lesions on the trunk.

improvement. This was 95% in group IV, 90% in group III, 85% in group II and 65% in group I. Complete remission was observed in 65% of the patients of group III, in 25% of group II, in 20% of group IV, and in 10% of group I. The difference between group III and the other groups as regards complete remission was statistically significant ($P<0{\cdot}01$; χ^2-test with Yates' correction). There was no significant difference with respect to the number of patients with good or excellent improvement between groups II, III and IV. The difference was almost significant between groups I and IV, in favour of the latter ($P<0{\cdot}02$; χ^2-test with Yates' correction). No real rebound phenomenon was noted after termination of treatment. During the 4-week follow-up period, 20–25% of the patients in all groups showed deterioration. At the end of the study 60% of the group III patients still showed complete remission.

Figures 2 and 3 illustrate the effect of the treatments on the extent of the lesions on the trunk and lower extremities, respectively. In group III there were more patients free of lesions on both

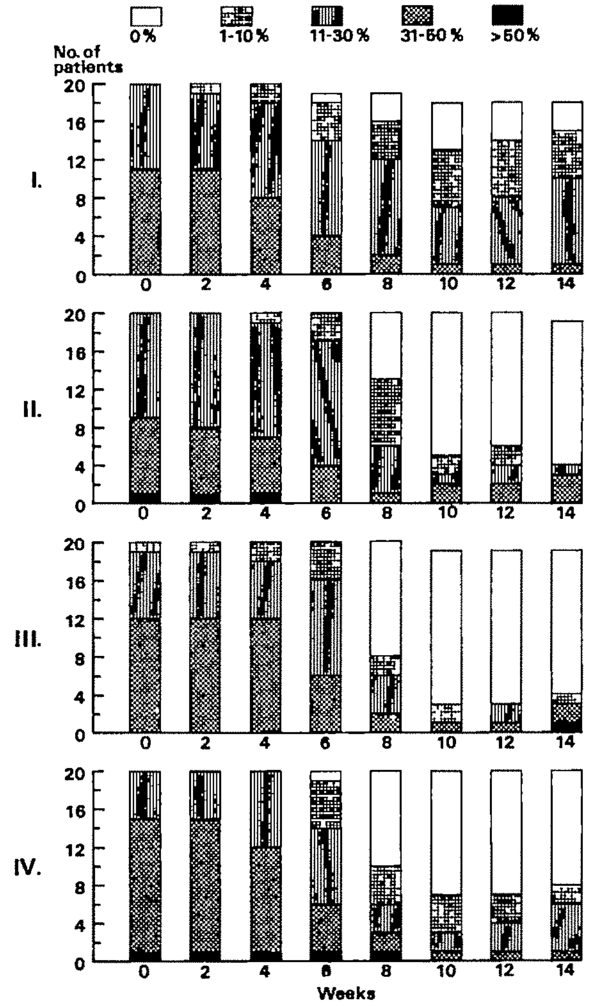

FIGURE 3. Extent of the lesions on the lower extremities.

TABLE 4. Total UVA doses (J/cm^2) and number of irradiations given to patients with good or excellent improvement during 10 weeks of treatment in groups II, III, IV. The figures are mean ± s.d.

Group	Total UVA dose (J/cm^2)	No. of irradiations
II	68·1±23·5	15·6±2·2
III	66·9±18.7	15·9±3·5
IV	199·5±46·8	26·5±3·9

TABLE 5. Adverse reactions during retinoid and RePUVA treatments

	Group I Retinoid	Group II RePUVA I	Group III RePUVA II
Erythema	14	8	12
Desquamation	12	8	10
Palmo-plantar desquamation	12	9	12
Dryness of the mucous membranes	13	12	14
Erosions of the mucous membranes	2	—	2
Conjunctivitis	—	2	—
Paronychia	1*	—	1
Sweating	2	1	—

* Treatment interrupted.

these skin areas as compared to other groups. Retinoid alone (group I) had less effect than the other treatments.

Table 4 presents the total UVA doses and the number of irradiations given to patients with good or excellent improvement in groups II, III and IV. The total UVA doses in groups II and III were about one third of those in group IV and there were about 40% fewer irradiations in groups II and III. The total UVA doses in groups II and III were highly significantly lower ($P < 0{\cdot}001$; Student's t-test) than those of group IV.

The adverse reactions recorded are shown in Table 5. In group IV the only side-effects were mild pruritus and nausea in a few cases. About two thirds of the patients in groups I and III who stayed on retinoid therapy throughout the 10 weeks showed at least once transient erythema and/or desquamation of their healthy skin. These side-effects were less frequent in group II (retinoid therapy for only 4 weeks). Dryness of the mucous membranes occurred equally in all the three groups in which retinoid was used. Other adverse effects rarely occurred.

A few patients of all four groups had mild, transient elevations in their serum transaminases. No abnormal elevations in alkaline phosphatase values were recorded during the study.

DISCUSSION

The results of the present study show that a combination treatment of oral retinoid and PUVA is very effective in clearing severe psoriasis. An advantage achieved by both the RePUVA modifications is a significant reduction of the UVA doses which may decrease the possible long-term risks of PUVA therapy. Our results are in agreement with those of Orfanos *et al.* (1978), Fritsch *et al.* (1978) and Heidbreder & Christophers (1979). The side-effects in our study were numerous but mostly mild and with one exception did not necessitate interruption of the treatment.

The type of therapy used in group III could be expected to be the most effective, since two different modalities with antipsoriatic activity were used simultaneously. However, the antipsoriatic action of neither treatment is as yet fully understood. Dierlich *et al.* (1979) found that orally administered aromatic retinoid has a distinct normalizing influence on epidermal proliferation, but with a latency of about 3 weeks. On the basis of this latency they suggested an extranuclear action which would also explain the favourable additive effect with therapies having primarily intranuclear activity. Supporting the concept of latency, Heidbreder & Christophers (1979) did not find any decrease in the number of mitoses during 2 weeks of pretreatment with retinoid. Retinoids have been found to inhibit the activity of ornithine decarboxylase (ODC), an enzyme essential for the biosynthesis of polyamines, known to be associated with cell growth and proliferation (Verma *et al.*, 1978; Weeks *et al.*, 1979). This provides a possible theory for the mechanism of action of retinoids. A cell cycle-specific locus of growth inhibition by the retinoids has been proposed by Haddox & Russel (1979), the target function being inhibition of ODC induction in the mid-G_1 phase of the cell cycle.

In RePUVA therapy, pretreatment with retinoid decreases desquamation and infiltration. PUVA treatment is then started in an improved state with less activity of the disease, which enables rapid clearing of the psoriatic lesions with low UVA doses. In previous studies the pretreatment periods have varied from 4–10 days (Fritsch *et al.*, 1978) to 2–3 weeks (Orfanos *et al.*, 1978). Because of the latency in the antiproliferative action of the retinoids it is obvious that maximal efficacy with minimal UVA doses is achieved if the retinoid pretreatment begins at least 3 weeks beforehand. On the basis of our experience we recommend a pretreatment period of 4 weeks which provides a good effect even when the retinoid administration is terminated before the start of PUVA treatment as in group II of the present study.

Besides the reduction of UVA doses there is another mechanism by which retinoid may enhance the safety of PUVA treatment. Naturally occurring retinoids are known to have an important role in the maintenance of normal cellular differentiation and to prevent the formation of premalignant squamous metaplasias on various epithelia (Mayer *et al.*, 1978). Retinoids also provide prophylactic and therapeutic activity in many chemical carcinogenic systems (Weeks *et al.*, 1979). Retinoids, therefore, may be able to inhibit malignant transformation possibly following high UVA doses. Some evidence of this protective action has been gained by the work of Epstein & Grekin (1979), who demonstrated an inhibition of UV carcinogenesis in hairless mice by topical application of retinoic acid in non-irritating concentration. Contradictory to this, earlier work had shown promotion of UV carcinogenesis (Epstein, 1977). In this work a severely irritating, toxic concentration of topical retinoic acid was used. The effect understandably is different when non-irritating lower concentrations are used or the drug is distributed to epidermal cells by oral administration.

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Case Report

Primary lymphoblastic non-Hodgkin's lymphoma of the skin

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SUMMARY

The clinical, histological, and ultrastructural features of a patient with a primary lymphoblastic non-Hodgkin's lymphoma of the skin are described. Four months after the onset of the disease the patient died with extracutaneous involvement of lymph nodes, spleen, liver, kidneys and lungs. The rapidly fatal outcome without the development of leukaemia or involvement of the bone marrow and the mediastinum is uncommon.

Primary malignant non-Hodgkin's lymphomas of the skin other than mycosis fungoides are very rare. In the Department of Dermatology of the Leiden University Hospital, this diagnosis has been made in only ten patients during the last 15 years. Retrospective analysis showed that one of these cases concerned a very uncommon primary lymphoblastic non-Hodgkin's lymphoma (Kiel classification: Gérard-Marchant *et al.*, 1974) of the skin. A review of the recent literature yielded only two reports of T-cell lymphoblastic lymphoma originating in the skin; both of these reports put the emphasis on the immunological and histopathological data, and paid little or no attention to clinical details (Nathwani, Kim & Rappaport, 1976; Berard *et al.*, 1978). Lennert (1978) reported fourteen cases of extranodal lymphoblastic lymphoma with neither T-cell nor B-cell characteristics (unclassified) occurring in the skin and subcutis, but in this report, too, clinical data are not given. In the present report we shall describe the clinical, histopathological, and ultrastructural findings in a case of a primary lymphoblastic lymphoma of the skin with an uncommon clinical course.

CASE-REPORT

In May 1974 a 61-year-old man developed bluish-red infiltrated lesions on both arms, some showing ulceration. A skin biopsy from one of the lesions was reported to show an extensive dermal infiltration of atypical reticulum cells. The diagnosis of reticulum cell sarcoma was therefore made.

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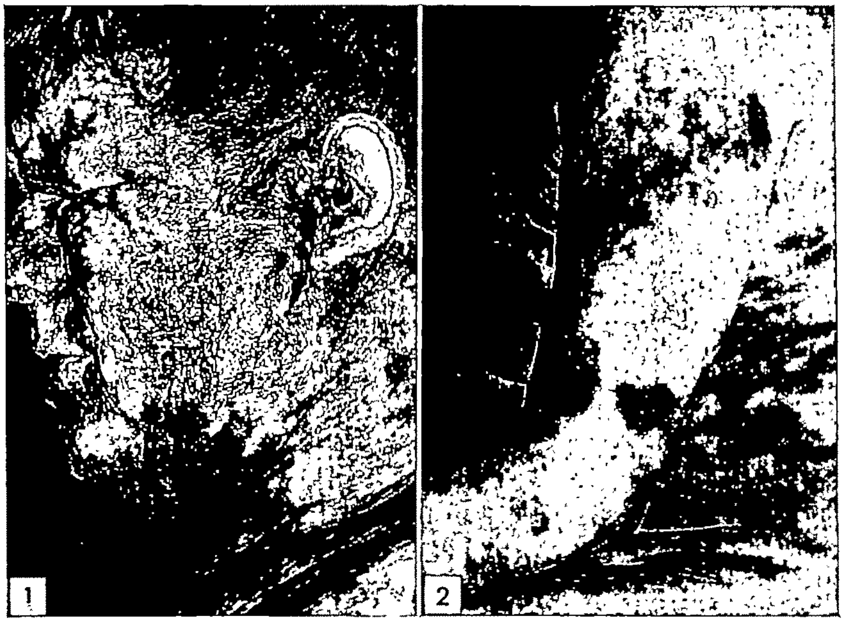
Because of progressive dissemination of the lesions, especially over the trunk and face, the patient was admitted for further investigation and treatment in July 1974. The past clinical history included chronic bronchitis, emphysema and a myocardial infarction in 1967.

Physical examination

Many bluish-red infiltrated plaques and tumours were present on the trunk, the arms and especially the face, producing the appearance of a 'leonine face' (Figs 1 and 2). Swelling of the eyelids made it almost impossible for him to see and because of the many infiltrated lesions around his mouth, he could hardly open it.

Except for a barrel-shaped chest and prolonged wheezing expirations, the general examination was normal; in particular there was no enlargement of the liver, spleen, or peripheral lymph nodes.



FIGURES 1 and 2. Infiltrated plaques and tumours on the face, producing the appearance of a 'leonine face' (Fig. 1), and on the left arm and trunk (Fig. 2).

Laboratory findings

The sedimentation rate was 14 mm/h, haemoglobin 15·3 g/100 ml, platelet count 284,000/mm^3, white blood count 10,200/mm^3, with the following differential: eosinophils 8%, neutrophils 65%, lymphocytes 26% and monocytes 1%. There were no atypical or immature lymphoid cells.

The liver function tests and the serum creatinine, urea, electrolytes, total protein, albumin and globulins all lay within normal limits.

Chest radiography showed no mediastinal mass and liver and spleen scintigraphy, lymphangiography and a bone marrow biopsy specimen did not reveal any indications of internal dissemination.

Treatment and course

The patient was treated with a combination of chlorambucil (10 mg/day) and prednisone (60 mg every other day), without response. Superficial X-ray therapy (100 rad, 125 kV) to the tumours on the face resulted in a marked reduction of the tumour mass, but after the cessation of radiotherapy these tumours recurred immediately. In this period, enlargement of the inguinal and axillary lymph nodes also occurred.

Because of the extent of the skin lesions and the insidious course, electron-beam irradiation of the face, as well as intermittent treatment with cyclophosphamide, vincristine and prednisone were started. On the first day of this treatment, early in September 1974, 4 months after the onset of the disease, the patient complained of feeling very tired, his temperature rose from 37·5°C to 38·5°C, and the pulse rate from 90 to 120/min. In the evening he became extremely dyspnoeic and died.

Histopathology

Review of the first skin biopsy revealed large sheets of medium-sized to large lymphoid cells with a moderately basophilic cytoplasm, reaching the subcutaneous fat. A distinct grenz zone was present in the upper dermis and no epidermal invasion by tumour cells was seen. Scattered between these cells there were prominent macrophages, often containing cell debris producing a 'starry-sky' pattern (Fig. 3). However, there were also areas without this pattern. The medium-sized lymphoid cells had round, often irregularly shaped (convoluted) nuclei, finely stippled nuclear chromatin, and inconspicuous nucleoli (Fig. 4). A number of tumour cells showed PAS-positive material in the perinuclear area. Mitotic figures were numerous. This histological picture is compatible with the diagnosis of malignant non-Hodgkin's lymphoma, lymphoblastic type. Skin obtained at autopsy showed the same histological picture as the skin biopsy.

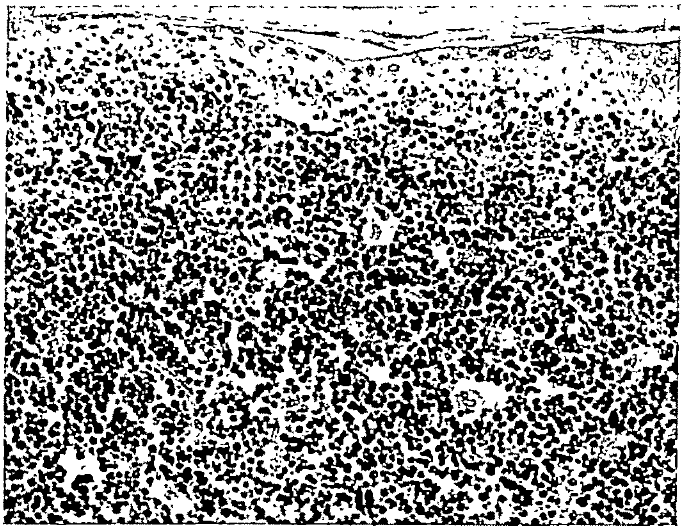


FIGURE 3. Micrograph of the dense dermal infiltrate, consisting of lymphoid cells, intermingled with prominent macrophages ('starry-sky' pattern) and separated from the epidermis by a distinct grenz zone (H & E, × 220).

To characterize the tumour cells more precisely, an indirect immunoperoxidase technique was used for the demonstration of kappa and lambda light chains, IgA, IgM, IgG and IgD heavy chains and lysozyme, after pretreatment of the paraplast embedded tissue sections with trypsin (Isaacson *et al.*, 1979). The tumour cells showed no intracytoplasmic staining for Ig. Weak lysozyme activity was found in the 'starry-sky macrophages'.

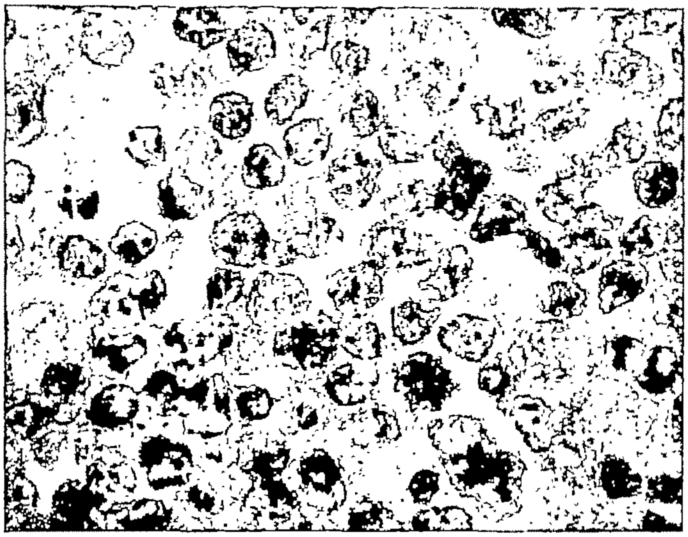


FIGURE 4. Detail of the dermal infiltrate, showing many lymphoblasts with round or irregularly shaped (convoluted) nuclei (Giemsa, × 2400).

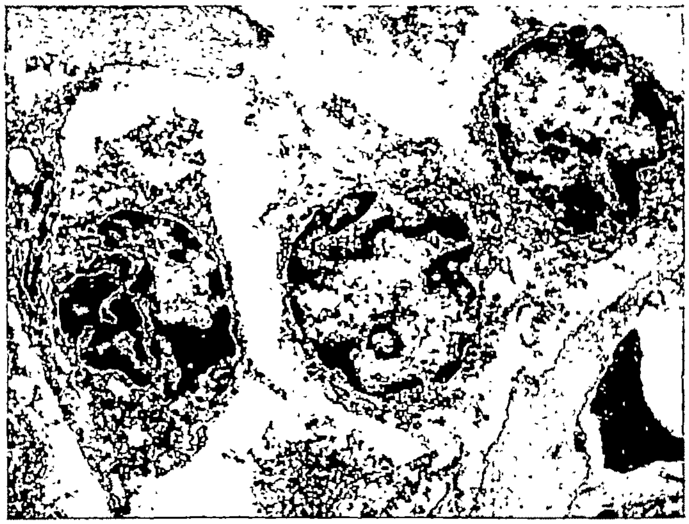


FIGURE 5. Electron micrograph of three lymphoblasts with irregularly shaped nuclei. Two tumour cells show deep invaginations (Formalin-fixed, paraplast-embedded skin tissue, secondarily embedded in Epon for EM, × 5800).

Paraplast-embedded tissue sections were stained for alpha naphthyl acetate esterase, alpha naphthyl butyrate esterase and acid phosphatase for at least 3 h. No enzyme activity could be demonstrated. Ultrastructural analysis was done on formalin-fixed paraplast-embedded skin, secondarily embedded in Epon. The results confirmed the convoluted nuclear structure (Fig. 5). Because of the poor primary fixation, preservation of cytoplasmic detail was inadequate and will not be further described. Despite the presence of tumour cells with convoluted nuclei, the tumour had to be classified as malignant non-Hodgkin's lymphoma, lymphoblastic unclassified, because the results of enzyme cytochemistry were inconclusive and immunological typing of the tumour cells was not performed.

Autopsy revealed tumour in cervical, axillary, mesenteric and para-aortic lymph nodes as well as in the spleen, liver, kidneys and lungs. Remarkably in these organs the 'starry-sky' pattern was seldom observed. Most of the lymph nodes had been totally replaced by tumour cells, but some showed partial involvement with a preferential localization of tumour cells in the para-cortical area. In these nodes, no primary or secondary follicles were seen. In the spleen the tumour cells were localized mainly in the red pulp. Local involvement of the white pulp, especially the peri-arteriolar lymphocyte sheath, was seen. In the liver, the portal areas showed a moderately dense infiltrate of tumour cells. In the kidneys, interstitial infiltration of tumour cells was found. In the lungs, tumour cells were localized along bronchi and bronchioli just under the epithelium in the lamina propria. No involvement of the hilar lymph nodes or the mediastinum was found at autopsy. The bone marrow too showed no tumour cells. Death was caused by haemorrhagic bronchopneumonia of the lower lobes of both lungs.

DISCUSSION

On the basis of morphological, clinical, cytochemical and immunological criteria, three main groups of lymphoblastic lymphoma (LBL) can be distinguished, namely B-cell LBL (Burkitt's type), T-cell LBL (convoluted type) and the unclassified LBL, including null-cell LBL (Kiel classification: Gérard-Marchant *et al.*, 1974; Lennert, 1978). In the present case, B-cell LBL can be excluded, because these are characterized by the presence of lymphoblasts with round or oval nuclei and prominent nucleoli. The irregularly shaped nuclei which often appeared to be convoluted, the absence of bone marrow involvement and the selective infiltration of the thymic-dependent paracortical areas in involved lymph nodes are a highly suggestive of a T-cell origin. However, paranuclear acid phosphatase activity, which according to Lennert (1978) is the most characteristic feature of T-cell LBL, could not be demonstrated, probably because only paraplast-embedded material was available. Furthermore, there is no clinical support for a T-cell LBL, because a mediastinal tumour, which is present in 50–80% of all T-cell LBL, was not found.

Since the T-cell origin of the tumour cells cannot be proven, the present case has to be placed in the group LBL, unclassified. With respect to the primary localization, most patients with T-cell LBL present either with an asymptomatic lymph node swelling, or with a mediastinal mass producing dyspnoea or dysphagia due to compression of the trachea or the oesophagus. In LBL, unclassified, a substantial number of patients present with, or rapidly develop, acute lymphoblastic leukaemia. The non-leukaemic cases often present with a localized tumour. Primary involvement of the skin is very rare in both types of LBL. Lennert (1978) described fourteen cases of LBL, unclassified, with localizations in skin and subcutis, but, because he gave no further clinical data, it is not clear whether primary or secondary involvement occurred. Berard *et al.* (1978) reported a case of a 60-year-old male with a T-cell LBL in whom a skin tumour remained the only localization for 2 years. After that the disease became leukaemic, as happens during the course of most cases of both T-cell LBL and LBL, unclassified, and neoplastic cells infiltrated the cerebrospinal fluid.

Nathwani *et al.* (1976), who compared two groups of LBL, one with and the other without convoluted nuclei, mention two cases with primary involvement of the skin. One patient with the 'non-convoluted nucleus type', in whom skin nodules were the first manifestation, died of leukaemia 2 months after the onset of the disease. The other patient, who had the 'convoluted nucleus type' presented with an extranodal mass in the thigh. He died 18 months later, apparently without involvement of the bone marrow or the peripheral blood. However, autopsy was not performed.

The rapidly fatal outcome of the present case is consistent with the literature. In most studies the median survival of LBL is less than 1 year (Nathwani *et al.*, 1976; Evans, Winkelmann & Banks, 1979).

In summing-up, this rapidly progressive lymphoblastic lymphoma of the skin differs from other lymphoblastic lymphomas with the same morphological and ultrastructural characteristics by the absence of a leukaemic phase and of involvement of the mediastinum and the bone marrow.

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Brief Communication

Alopecia areata regrowth induced by *Primula obconica*

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Hair growth has been induced in a case of alopecia areata by rubbing lightly with *Primula obconica* leaves.

The patient was first sensitized to primula by wearing a leaf continually against the skin, changing this for a new one every week. Sensitization in her case took about 6 weeks. Afterwards she applied a leaf to one side of her scalp, at first nightly, but as this produced too fierce a reaction, then twice a week. Hair growth started about 1 month later and was quite evident 2 months later (see Fig. 1).

We have since used *Primula obconica* leaves in four other patients and produced a response in all of them. Blistering has been a problem but the patients soon became wary of too enthusiastic applications. Obviously a cream or gel containing suitable concentrations of primin would be the answer and we are working on this.

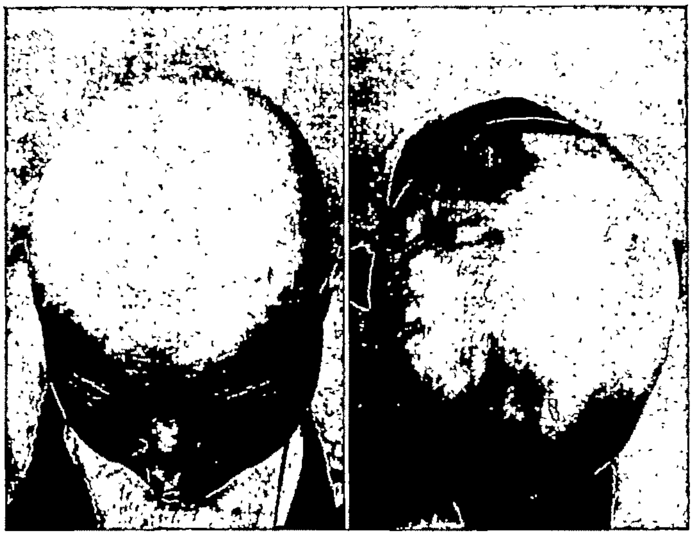

FIGURE 1. Hair growth induced by Primula obconica. (left) Before treatment. (right) After treatment.

Corticosteroids have been used to induce hair growth in alopecia but the primula applications seem much more effective. Irritants such as ultraviolet light, liquid nitrogen and dithranol (Schmoekel & Weissman, 1979) have also been used and presumably induce hair growth by increasing the blood supply to the hair follicle. Dinitrochlorbenzene as a sensitizer is effective (Daman, Rosenberg &

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Drake, 1978; Frentz & Eriksen, 1977), but this substance is a mutagen and its use in the U.S.A. is falling into disfavour. Alternatives such as poison ivy are being tried but it would seem unwise to sensitize a patient to an antigen which might be met with in everyday life. *Primula obconica* can easily be avoided.

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Comment

CHANGING CONCEPTS IN MELANOMA MANAGEMENT

'We have seen a number of melanomas where the base of the tumor *seemed* to form *almost* a straight line and impinge upon the upper part of the reticular dermis without showing any *significant* invasion of the reticular dermis' (Clark *et al.*, 1969).

The above quotation (italics by the author) represents the scientific basis of the microstaging system which has ruled the world of human cutaneous melanoma for over a decennium. In the seventies the name of Clark has been used almost eponymously with malignant melanoma. However, evidence is now arising that his concept of five levels of invasion is an inaccurate measure of prognostication and, therefore, an imprecise guide to melanoma management. The (anticipated) abandoning of Clark's microstaging method is one of the most remarkable breakthroughs in the melanoma field. This review will be concerned with some important changing attitudes in the clinical approach to primary cutaneous melanoma. Lack of space precludes a comprehensive discussion of all aspects; therefore the reader is referred to other recent reviews (Davis, 1976; Kopf, Bart & Rodriguez-Sains, 1977; Goldsmith, 1979a; Lee, 1980). Also, the current thinking with regard to chemo- and immunotherapy of melanoma is beyond the scope of this appraisal.

PROGNOSTIC FACTORS

A plethora of variables which influence prognosis in malignant melanoma has been reported. The more important of these factors, arbitrarily grouped into four categories, are summarized in Table 1. An accurate appreciation of prognostic criteria is a *sine qua non* for the choice of appropriate treatment procedures.

Stage

The staging of malignant tumours depicts the progression of the extent of the disease, which is a function of time. According to the TNM-system, melanoma patients are allotted to particular categories, based on the assessment of three components: T, the extent of the primary tumour, N, the condition of the regional lymph nodes, and M, the absence or presence of distant metatases. Whereas previous TNM-classifications of malignant melanoma grouped the primary tumour according to maximum diameter, recent staging systems correctly appreciate the microstaging techniques described by Clark *et al.* (1969) and Breslow (1970). Unfortunately there are at present in vogue numerous TMN-staging methods which are far from uniform. Fresh efforts should be made to arrive at an international agreement upon the staging of cutaneous melanoma, in order to facilitate the exchange of treatment data between various centres.

Tumour characteristics

The classification of malignant melanoma into histogenetic types as proposed by Clark *et al.* (1969) is crucial: the intermingling of lentigo maligna melanoma (LMM) with superficial spreading melanoma (SSM) and nodular melanoma (NM) in survival statistics appears unjustifiable, since the site

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TABLE 1. Prognostic variables in malignant melanoma

(A) *Stage of the disease*	Maximum diameter Level of invasion (Clark *et al.* 1969) Tumour thickness (Breslow, 1970) Regional metastasis Distant metastasis
(B) *Tumour characteristics*	Histogenetic type Mitotic rate Vaso-invasive properties Amelanosis Ulceration Tumour shape (cross-sectional profile) Evidence of partial regression Satellitosis
(C) *Host characteristics*	Sex (hormonal status) Age Anatomical site Inflammatory reaction around primary
(D) *Iatrogenic factors*	Maltreatment (incisional procedures)

and age distribution, as well as the biological behaviour of LMM indicates that this type forms a distinct entity. The differentiation between SSM and NM is less important since tumours of comparable microstages have similar mortality rates, regardless of which type they are (McGovern *et al.*, 1979). The designation 'acral lentiginous melanoma' (ALM) was coined by Clark *et al.* (1975) for melanomas of the palms and soles and subungual locations. Since the clinical and histological characteristics of ALM are different from those of the other histogenetic types, it is recommended that the latter category be treated separately.

Mitotic rate is a reliable criterion of prognosis. Schmoeckel & Braun-Falco (1978) reported that the most valid index of prognosis can be derived from the product of tumour thickness and mitotic rate. Whether the mitotic activity is an independent variable or not is a matter of controversy. The papers of Larsen & Grude (1979) and McGovern *et al.* (1979) indicate that the predictive value of mitotic count generally correlates with lesion thickness or the level of invasion. The same applies to other tumour characteristics like vaso-invasive properties (Larsen & Grude 1979) and amelanosis (Balch *et al.*, 1978). Apparently the malignant potential of melanoma increases with the tumour volume. This phenomenon can be plausibly explained by the multiclonicity of tumours in general (Poste & Fidler, 1980). Although malignant growths probably arise from single cells, sooner or later they become multiclonal. Different cell-lines usually co-exist within the same tumour. Conceivably, the more 'malignant' substrains ultimately prevail over the less aggressive ones. This may also explain the findings of Barr, Gartside & Goldman (1979), who observed behavioural differences, as expressed by the capacity of transplanted tumour cells to grow in the nude mouse and by the incorporation of tritiated thymidine, between cell-lines from 'low-risk' and 'high-risk' primaries and from lymph node metastases.

Amelanosis probably indicates the dedifferentiation of the melanoma cell which may partly explain differences in prognosis. On the other hand, amelanotic lesions are easily misdiagnosed and doctors' (and patients'!) delay is apt to occur. Ulceration (Balch *et al.*, 1980) and tumour shape or cross-sectional profile (Beardmore, 1977) are recognized features influencing survival. Both variables are interrelated since heavy protrusion of the tumour promotes traumatization, resulting in ulceration.

Polypoidal melanomas which frequently show evidence of ulceration have a notoriously dismal outcome. Trivial injuries may cause shedding of viable cells from the primary and subsequent lodgement of these cells at distant sites. The same mechanism is supposed to be involved in iatrogenic traumatization through incisional diagnostic procedures (*vide infra*).

Areas of regression within a malignant melanoma paradoxically represent a determinant of relatively high risk (Gromet, Epstein & Blois, 1978). Though regression may signify a host immune response to the tumour, this finding seems to correlate with a poor prognosis. However, McLean *et al.* (1979) found that the clinical appearance of white depressed areas, which most likely represent areas of spontaneous regression, was not of prognostic importance. Similarly, McGovern *et al.* (1979) failed to confirm the adverse influence from partial regression upon survival. Regressing lesions displayed a slightly superior prognosis which was attributable mainly to the correlation with tumour thickness.

Host characteristics

Sex differences in the survival from melanoma have been ascribed to the more favourable location of the tumour in women (predominantly the leg) as compared to men (mainly the trunk) and to the earlier stage of the disease at first presentation (Shaw *et al.*, 1978). Recently, Rampen (1980) and Rampen & Mulder (1980) showed that prognosis favoured female survival, irrespective of age, site or stage. Measuring the tumour volume doubling time of pulmonary metastases, it was shown that women exhibited a slower growth rate of their secondaries than men. This may be accounted for by hormonal influences and it is tempting to speculate that malignant melanoma represents an androgen-dependent tumour. Other factors, however, may also play a role. For instance, smoking habits may affect metastasization by increasing the blood viscosity (Shaw *et al.*, 1979; Dintenfass, 1979).

Incisional biopsies

Complete surgical removal is generally advocated as the first diagnostic and therapeutic approach for any suspicious lesion. Whether incisional procedures have a bearing on prognosis has long been a matter of dispute. Epstein, Bragg & Linden (1969) emphasized that biopsy prior to definitive treatment did not decrease the survival rate. However, since their biopsy category comprised incisional as well as excisional biopsies, no valid conclusions pertaining to the detrimental influence of incisions on survival can be generated from this study. The paper of Rampen, van Houten & Hop (1980) persuasively assessed the deleterious effect from pretreatment incisional biopsies on prognosis. A sketchy account on the same subject was reported by Van Slooten & Van der Esch (1977). To test the theory of facilitation of tumour cell detachment from the primary during surgical manipulation, B16-melanoma-bearing mice were subjected to either amputation of the leg after incisional biopsy of the tumour on the footpad ($N = 17$), or to amputation alone ($N = 11$): pre-operative incision significantly reduced the survival time ($P = 0{\cdot}01$) (Mulder, Rampen & Van der Lugt 1978). These data demonstrate an increased risk of tumour cell dissemination after physical trauma. Therefore, whenever an incisional biopsy is considered within the framework of a diagnostic procedure, this hazard should be taken into account.

CLARK VERSUS BRESLOW

Mehnert & Heard (1965) have the credit for introducing a system of four levels of micro-invasion: intra-epidermal (*in situ*) melanoma, melanoma with invasion into the papillary dermis, into the reticular dermis, and into the subcutaneous structures. Mortality rates were proportional to depth of invasion. Later, Clark *et al.* (1969) modified this system by introducing a special 'level III' melanoma in which tumour cells accumulate along the interface between papillary and reticular dermis.

In 1970 Breslow showed that tumour thickness, as measured with an ocular micrometer, provides an objective method of predicting prognosis. Evidence is arising that the level of invasion is an indirect measure of tumour thickness. Balch *et al.* (1978) found that maximum tumour thickness is a more powerful parameter than level of invasion. The same conclusion was reached by the WHO Melanoma Study Group (1978). The pros and cons of the two histological parameters have been reviewed excellently by Breslow (1979). The major disadvantage of Clark's system is the subjective description of 'level III' melanoma. Whereas the other levels of invasion indicate infiltration through a defined volume, 'level III' melanomas seem to be temporarily arrested at the papillary-reticular interface, impinging upon the reticular dermis (Fig. 1). No valid explanation has ever been advanced for this non-belligerent episode in the development of human cutaneous melanoma.

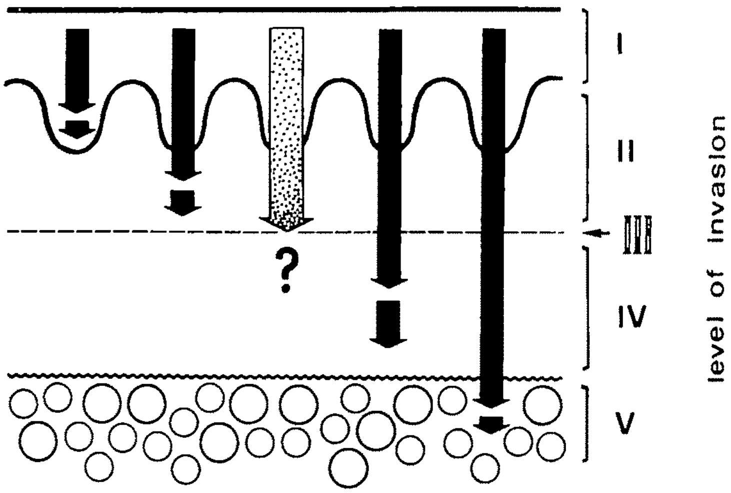

FIGURE 1. The Clark 'level III' melanoma; does it exist?

In all likelihood, the creation of the 'level III' melanoma has issued from the difficulties sometimes encountered in the identification of the junction between papillary and reticular dermis. Moreover, the tumour-associated lymphocyte infiltration around the melanoma may interfere with the accurate distinction of the interface. Therefore, Clark's method appears to be founded on vague premises, and it is not against expectation that several leading centres in the world have already abandoned using this system. If depth of invasion according to anatomical strata is to be recorded at all, it probably suffices to use the criteria proposed by Mehnert & Heard (1965).

The subjectivity of Clark's staging is further underscored by the unsatisfactory overall agreement between pathologists in a recent congruence survey on the classification and grading of histological criteria (Larsen *et al.*, 1980). Consistency was least in the level of invasion compared to four other histological parameters. Agreements below 67% were made in 50% of all slides.

SURGICAL MANAGEMENT

Primary treatment of malignant melanoma consists of wide three-dimensional excision, preferably under general anaesthesia. Controversy exists concerning the width of local excision. The question of prophylactic lymphadenectomy forms a similar disputable issue.

Resection margin

Many reports have expounded upon the necessity of an optimal resection margin of about 5 cm beyond the perimeter of the lesion. Cosmetic or functional considerations may influence the extent of excision (face, hands and feet). Recently it was shown that tumour thickness is a useful parameter for the width of the tumour-free resection margin (Breslow & Macht, 1977). For thin melanomas, excellent survival was reported even with very narrow resection margins. Similar results were obtained by Balch *et al.* (1979). At present, a randomized trial on the evaluation of the optimal resection margin in melanomas thinner than 2 mm is being carried out by the WHO Melanoma Study Group.

Elective regional lymphadenectomy

There is little disagreement among clinicians with regard to the unjustifiability of elective lymphadenectomy in 'low risk' melanoma patients. On the other hand, considerable confusion exists as to whether non-palpable regional lymph nodes should be routinely resected in 'high risk' patients, i.e. patients with melanomas of advanced Clark-Breslow microstages. Many retrospective studies have not settled the controversy. To date there are only two randomized prospective trials of elective lymphadenectomy for stage I melanoma (WHO Melanoma Study Group, 1977; Sim *et al.*, 1978). Both studies failed to substantiate any benefit from prophylactic dissection. However, in the Mayo Clinic series (Sim *et al.*, 1978), 64% of the patients had melanomas less than 1·5 mm thick and only 7% of electively removed nodal areas contained occult secondaries.

With reference to the WHO study (1977), it is emphasized that 5-year survival rates for patients with immediate prophylactic lymph node dissection and for patients with delayed therapeutic dissection were very similar. Yet, various subgroups showed an apparent, though not statistically significant, benefit from elective lymphadenectomy, for example Clark level IV lesions and lesions of 1·6–4·5 mm thickness. In this respect, the exhaustive and critical review of the WHO study by Goldsmith (1979b) is noteworthy. Further scrutiny of the WHO data reveals other interesting facets. The risk of multi-institutional randomized trials becoming multi-clinician trials (Williams & Whitehouse, 1979) is illustrated by the unbalanced numbers of patients allocated to the trial from various cooperating centres: Milan, eighty-three, several Eastern-European centres, about fifty each, against Oslo, eight and Naples, five (WHO Melanoma Study Group, 1976). Secondly, the lack of comparability with regard to sex and maximum diameter between the two groups may have biased the results (Table 2). One wonders if the selection and randomization procedures have been carried out

TABLE 2. Distribution of patients according to their sex and the maximum diameter in the two treatment categories of the WHO study on elective lymphadenectomy (1977)

		Number of patients		
Prognostic indicator	Variable	Excision only	Excision + elective node dissection	Significance*
Sex	male	46	57	$\chi^2 = 2{\cdot}2$
	female	240	210	N.S. ($P > 0{\cdot}10$)
Maximum diameter	≤10 mm	100	64	$\chi^2 = 11{\cdot}4$
	11–20 mm	118	119	$P < 0{\cdot}005$
	>20 mm	35	54	

* χ^2-test with Yates' correction; N.S. = not significant.

correctly. What is more, the provocative statement emerges that the WHO study provides evidence in support of the proponents of prophylactic node dissection; despite the preponderance of female patients and of tumours of small size in the 'excision only' category, the survival curves of both groups were identical. This can only be explained by the favourable influence of elective lymphadenectomy in the node dissection group.

PROGNOSTIC INDEX

The assessment of prognosis in cutaneous melanoma using a prognostic index was first described by Cochran (1968). Cochran's system retrospectively attributed certain score values related to clinical and pathological features to each tumour. The prognostic index was obtained by totalling the various scores. Later, Cochran's system was validated by Barclay *et al.* (1977). A different approach was launched by Schmoeckel & Braun-Falco (1978) who corroborated that a more sophisticated index is derived by the product of tumour thickness and mitotic rate. The latter method appears to be more objective than Cochran's index. However, since numerous factors have a bearing on the prognosis for melanoma, prognostication based on two variables only is an unwarranted simplification. The application of appropriate multifactorial statistical models like the one proposed by Cox (1972) may result in a refinement of the inference of Schmoeckel & Braun-Falco (1978), giving weight to several independent prognostic indicators, including the stage of disease, tumour as well as host characteristics, and iatrogenic factors like maltreatment.

Even then, the 'ideal' prognostic index does not exist. Aiming at predicting metastatic spread, prognostic score sheets should allow clinicians to select high-risk patients for adjuvant therapy modalities, for instance prophylactic node dissection, regional perfusion, or chemotherapy. So far, literally no information exists on the relative risk for haematogenic against lymphogenic metastatic potential of any of the previously cited prognostic indicators. For instance, lymphatics reportedly are absent in the upper dermis of normal skin (Ryan, 1978). Consequently, melanomas with invasion limited to the papillary dermis probably metastasize via the haematogenous route only. Therefore, very thick melanomas with superficial invasion (e.g. polypoidal non-stageable melanomas), bearing an unfavourable prognosis, would be best submitted to elective chemotherapy instead of regional approaches. Another example is the difference in pattern of metastatic spread between male and female patients: initial systemic relapse occurs predominantly at visceral locations in males, whereas females exhibit relatively frequently initial distant spread at cutaneous or nodal sites, indicating a female preference for lymphogenic metastasization (Rampen & Mulder, 1980). If in future studies this preference can be born out, the female sex then constitutes a risk factor with respect to prophylactic regional lymphadenectomy or perfusion therapy, in contrast to males who form a subgroup of patients who would benefit most from chemo- or immunotherapy. These and other subtleties await further elucidation before a rational basis can be reached with a view to prophylactic management of cutaneous melanoma. At the moment such procedures are instituted almost haphazardly, a fact of considerable importance since all prophylactic treatments bear a not negligible morbidity.

Obviously, many combined efforts of clinicians, pathologists and tumour biologists are necessary in order to arrive at more sophisticated treatment schedules for melanoma patients at risk. The rejection of Clark's microstaging method (Clark *et al.*, 1969) and of the WHO study on elective lymphadenectomy (WHO Melanoma Study Group, 1977) would make a good start.

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Pyoderma gangrenosum and haematological malignancies

SIR, In a reappraisal of systemic diseases associated with pyoderma gangrenosum, Hickman & Lazarus (1980) have made the following statement: 'a clinically distinctive more superficial bullous variety of pyoderma gangrenosum may occur with haematological malignancies, including blast crisis of polycythaemia vera or myeloid metaplasia, acute or chronic myelogenous leukaemia, and acute lymphoblastic leukaemia.' As the only reference for this statement they have given an article by Perry & Winkelmann (1972) on pyoderma gangrenosum and leukaemia in which none of the cases reported was associated with polycythaemia vera. I should therefore like to note that in 1971 we published two papers on the association of pyoderma gangrenosum with polycythaemia vera (Feuerman & Potruch-Eisenkraft, 1971) in which we were able to cite a number of other articles also reporting on this association (Basset, Maleville & Bergoend, 1969; Bureau *et al.*, 1967; Duverne *et al.*, 1967). The number of cases reported in the literature, as well as those which undoubtedly have remained unreported, strongly support my own feeling that this association is not merely coincidental.

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Nicotine: an hapten

SIR, In the Review Article 'Tobacco dermatitis' by R.J.G. Rycroft in the *British Journal of Dermatology* (1980) **103**, 225, the author writes that 'nicotine itself is not the sensitizer (Silvette *et al.*, 1957)'.

We have previously studied a familial dermatitis (Sudan & Sterboul, 1979) which resembles seborrhoeic dermatitis. In all patients, the *in vitro* human basophil degranulation test (HBDT) (Baso-kit, procede Jaques Benveniste, Laboratiore des Stallergènes, Paris), a test developed by J. Benveniste (Benveniste, 1978), gave positive results when using either the allergen tobacco (leaves, Hollister-Stier: >50% from 10 μg to 10^{-1} μg) or the hapten nicotine (>50% from 1 μg to $10^{-2}\mu$g) bound to human serum albumin (HSA). RAST (Radioallergosorbent test, Pharmacia, Uppsala) results were positive for the first author (B.J.L.S.) with allergenic extracts of tobacco leaves: 4 + (tobacco leaf A), 3 + (tobacco leaf B) and 3 + (tobacco leaf C). These results were in accord with a familial and personal history of atopic reactions and suggested that the skin mast cells (by virture of their capacity to release the chemical mediators of anaphylaxis) were the cause of the clinical symptoms. Topical sodium cromoglycate (SGG: 5%) (Loniudal, Lonsusol, Welcrona, Fisons Laboratories) seems to be of benefit in this reaction (Sudan & Sterboul, 1980).

We have recently studied a new case of sensitization to the nicotine alkaloid of *Nicotiana tabacum*. Mr Joseph B., aged 32 years, was afflicted with a facial dermatitis in December 1978. This reaction had been recurring regularly since puberty and corresponded exactly to that of seborrhoeic dermatitis. There was no personal or familial history of atopy. The allergic investigations revealed positive intracutaneous tests read at 15 min to tobacco extracts (leaves; Hollister-Stier). The provocation test by inhallation of tobacco smoke showed strong positive results. The level of total IgE antibodies by RIST (Radioimmunosorbent text, Pharmacia, Uppsala) was normal; however, the level of eosinophilia was 7%. The HBDT test showed strong positive results when using the allergens tobacco (leaves, Hollister-Stier: 66%; 100μg) and nicotine-HSA (58% 10 μg).

Avoidance of tobacco smoke has cleared the dermatitis in all patients. Thus there is a cellular reaction to tobacco antigens detectable in a syndrome of unknown aetiology classified for a century (Unna, 1887) as 'seborrhoeic dermatitis', which first appeared at about the time that tobacco smoking was becoming widespread in public places.

ACKNOWLEDGMENT

We thank Professors Samuel B.Lehrer (Tulane University, Clinical Immunology Section, New Orleans, U.S.A.) for RAST assays and Jacques Benveniste (I.N.S.E.R.M. U 200, Immunopharmacologie de l'allergie et de l'inflammation, Clamart, France) for HBDT test.

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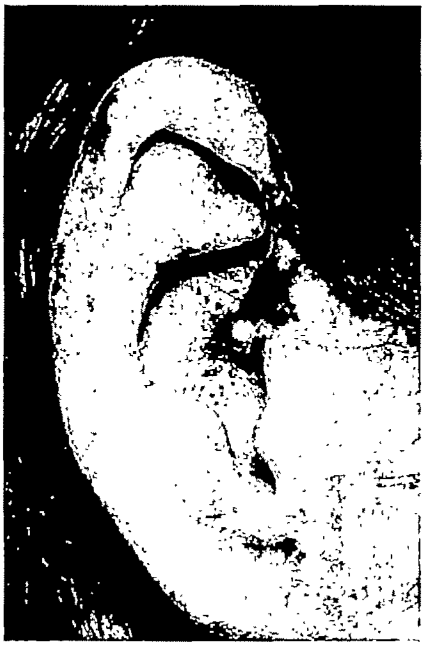
A simple sign of discoid lupus erythematosus

SIR, I would like to record a simple physical sign the only merit of which is its existence (see Fig. 1).

I have found that about one in three patients with discoid lupus erythematosus have diagnostic changes on the lateral surface of the pinna. They occur mostly but not exclusively in the concha and triangular fossa and consist of wide follicular pits many of which house a scale or blackhead. The pits usually show no signs of inflammatory activity and those without a scale are, I presume, atrophic remnants of the disease. The more inflammatory lesions are usually larger and ultimately subside into the well-known irregular cliff-edge depressions. I now look for the sign regularly and in recent years have found it in about a dozen or so patients: I have never found it other than in discoid lupus erythematosus but I would not be surprised to find it occasionally in patients with lichen planus and lichen



sclerosus. Although the histological appearance of the lesions and their relationship to age, sex, severity and what you will might be worth further study, the sign itself has never helped me make a diagnosis other than by strengthening the resolve of a weak nature.

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Herpes Zoster

SIR, In the *British Journal of Dermatology* (1980) **102**, 551, 'Do corticosteroids prevent post-herpetic neuralgia?', Keczkes and Basheer, in their open study, presented a favourable treatment with the use of prednisolone. May we present our preventive therapy for the same complication, with adenosine 5′-monophosphate (AMP), an ubiquitous natural cellular key metabolite which is the precursor of many essential cellular metabolites.

The use of AMP as a favourable treatment for the control of recurrent herpes labialis (Sklar & Klein, 1979) indicated that this safe compound might be extended to the treatment for the prevention of post-herpetic neuralgia.

It was demonstrated (Sklar, 1977) that the blood nucleotide levels of AMP and adenosine triphosphate were abnormally altered in patients with herpes zoster and herpes simplex infections. The blood nucleotide values of AMP, employing the methods of Jaworek, Gruber & Bergmeyer (1974), were found to be consistently low (normal, 0·5–1·2 mg/dl) and conversely adenosine triphosphate nucleotide levels were abnormally high (normal, 19–32 mg/dl). Moreover, the use of AMP therapeutically returned blood values to normal levels commensurate with the patients' recovery.

Over a two-year period, in an open preliminary clinical study of over 130 patients with early onset of herpes zoster, all were referred by their physician for treatment with AMP. Table 1 presents the age distribution.

TABLE 1. Herpes zoster cases distribution by age in years

Age	No. of cases treated
7–9	1
10–19	5
20–29	4
30–39	3
40–49	11
50–59	33
60–69	30
70–79	25
80–89	18
90–95	1
Total	131

Adenosine 5′-monophosphate, 1·5–2·0 mg/kg, was given intramuscularly on alternate days for 12–15 injections. Erupting vesicles were arrested with little confluence. Discomfort, general malaise and severe pain were reduced, with rapid progressive drying and desquamation of the lesions. The patient, depending on the severity of the disease, usually recovered completely within 3–4 weeks of therapy, being able to return early to his gainful occupation with no evidence of post-herpetic neuralgia. Twelve patients mentioned a lingering discomfort, but no pain, in the involved area, which disappeared in 1–2 months. In an observed 2-year period, there was no recurrence of pain.

An available explanation cannot be offered regarding the effect of AMP with herpes zoster. It is difficult to decide whether the rapid improvement in patients is due to an undemonstrated molecular repair of the nerve cell within the ganglion or modification of the virus induced by AMP or eradication of the latent virus from the ganglion. Our holistic approach to therapy considers maintaining cellular integrity with regard to its function and dynamics.

Clinically, this preliminary study indicates that the use of AMP therapeutically may hold a place in the armamentarium for the potentially effective preventive treatment of post-herpetic neuralgia. Nevertheless, our forthcoming formal controlled study, we believe, should confirm this preliminary observation. AMP has been employed safely in both children and adults. No presenting toxic or side effects have been reported or demonstrated with its use.

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Aryl hydrocarbon hydroxylase activity in psoriasis

SIR, Mier and his colleagues suggest (Mier, Commans & Roelfzema, 1980) that the unique defect of aryl hydrocarbon hydroxylase (AHH) activity in the clinically normal epidermis of patients with psoriasis

(Chapman, Rawlins & Shuster, 1979; Shuster *et al.*, 1980) might be confined to the Langerhans cell. As might be expected of a fundamental genetic defect (Shuster *et al.*, 1979) we have found the abnormality of AHH in all cell types so far examined in psoriasis (Chapman *et al.*, 1980). In the epidermis itself we have found that almost all the measured AHH activity is in the basal cells (in preparation) and not surprisingly, therefore, epidermal AHH persists in skin in which the Langerhans cells are destroyed by UV irradiation (in preparation). Whilst this does not exclude a role for the Langerhans cell in psoriasis it does break the arm of coincidence upon which Mier's hypothesis rests. The crunch line is how a change in AHH activity is translated into an increase in cell production and the lesions of psoriasis (Shuster *et al.*, 1980) and at this stage we feel safer with a herd of hypothetical horses which we can change mid-stream, than with the confines of this year's cell of the moment.

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Reply

SIR, We have read with interest the latest findings of the Newcastle team. These results (if and when confirmed) would indeed seem to weaken the case for a monocyte-Langerhans cell genesis of psoriasis.

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British Journal of Dermatology (1981) **104**, 355.

Book Reviews

Recent Advances in Dermatology—5. Edited by ARTHUR ROOK and JOHN SAVIN (1980). Edinburgh: Churchill Livingstone. Pp. 352. Price: £18.00.

I was very pleased to be asked to review this book because it is one to which I shall gladly give shelf-space once my task is done, and I do not doubt that subsequently I shall often take it down and read it.

The reasons for referring to a book like this will obviously vary from one person to another but most will do so with expectations of filling in gaps outside their immediate field painlessly and with confidence that the material with which they are filling them is right. A choice of 'best' chapters will also be a personal one depending partly on personal gaps, interests and background experience; indeed a list of our colleagues' favourite chapters may tell us almost as much about them as about the quality of the chapters: for the record my favourites were those on cutaneous immunopathology, mycosis fungoides and the kinetics of epidermal cell populations.

The whole book is very readable, attractively produced, a good source of references and well indexed. The list of contributors includes such dermatological and other 'heavyweights' as Jordon, Mackie, Touraine, Winkelmann, Farber and Wright, and when one adds the editors themselves to this list it is hard to see how the book could fail, as indeed it does not.

In any multiple author book about recent advances where specialists write about their own subjects, one expects an assessment of the advances as well as a statement that they have taken place. Generally one gets this here. It is not the author's fault that the chapter on the HLA system in dermatology soon degenerates into a list of diseases alongside a list of preferred HLA types. Calculations show of course that the associations are statistically significant but with few exceptions they are not clinically helpful and even their scientific interest is waning: perhaps by the time the next Recent Advances is published the HLA message for dermatologists will have crystallized. The chapter by Ive and Comaish reminds us that advances in dermatological treatment in the past 5 years have not kept pace with those in other fields and their assessment is that even a number of promising lines 5 years ago did not stand the test of time.

The book at £18 is worth the money to those clinical and academic dermatologists who are in established practice or in training: it also contains enough of general interest to make it a good investment for some non-dermatologists.

JANET MARKS

Common Skin Disorders. A Manual for Physicians and Patients. ERNEST EPSTEIN (1979). Oradell, N.J.: Medical Economics Company Book Division. Pp. 272. Price $14.95.

Education in understanding the nature of common skin diseases and in their management has spread in recent years from an interprofessional matter to the field of 'popular science'. Some may still regard this as the vulgarization of an art, but all of us must accept that there is an increasing patient demand for more detailed information than we have been used to providing. In this country the demand is still less exacting than in the States but is continually bolstered by the media and Consumer Groups and Societies. The patient's (or parent's) understanding of atopic dermatitis, for instance, is usually confused and basic management suffers thereby; 'sensitization' is a concept that few people appreciate.

Ernest Epstein has brought together short and concise explanatory sections, chiefly concerned with the practical treatment and management of common skin disorders and therapeutic situations and has linked these with 'Patient Instruction Sheets', which are even shorter. Dr Epstein's high reputation as a practical physician ensures that the information given is sensible and 'down-to-earth'. Few British dermatologists would quibble with his recommendations—though there are inevitably some differences of approach and detail. Few of us in this country would, I think, suggest prednisone (in a starting dose of 40 mg/day) for pityriasis rosea, even if this condition does make the patient 'uncomfortable'. But such national and cultural differences are acceptable.

The Instruction Sheets are inevitably orientated towards private practice and the direct and continuing involvement of the specialist. The repeated advice to 'phone my office' if such-and-such occurs would probably not go down well with all of us—though it might prevent a few sleepless nights. But whether we use Instruction Sheets or not, this little book should be read by all dermatologists, for it is full of wisdom and the practical fruits of a long experience by a physician who cares for his patients as much as for his art.

D.S.WILKINSON

Laser Treatment for Naevi. KOSHIO OHSHIRO (1980). Tokyo: Med. Laser Research Co. Ltd. Pp. XV + 149 + XXVI, 234 illustrations, 185 in colour. Price Y20,000.

This book aims to be 'an interdisciplinary introduction to an interdisciplinary field'. The author, an internationally known innovator and expert in the field, does not purport to have produced a manual or workbook of laser therapy.

It is divided into two sections: theoretical and practical. The first part, one third of the text, sketches the medical and physical background of naevi and lasers respectively. Both subjects are dealt with in a very basic way, assuming the reader has very little knowledge. The practical section outlines the capabilities of the different types of laser; following this is the main part of the text, 45 pages of script accompanied by colour plates. It is divided into subsections giving examples of therapy for each of the naevi considered. Each lesion is described and illustrated with a high quality photograph. The type of laser used, together with the dose applied, is stated but with no discussion as to why a particular combination was chose. The before-and-after photographs show the very good results which can be obtained with laser therapy but leave one to speculate about the failures—how many strawberry naevi failed to respond or reacted adversely? One must say that the frequent comparisons with conventional techniques are more than a little hard on the latter. The last section is a useful glossary of terms and references.

This is a beautifully produced and instructive book which gives a broad insight into the current position of lasers, but it is rather expensive, unnecessarily long and could easily have been halved in size, and cost. The 13 pages showing other modes of treatment (often only two colour plates per page), the space wasted in the reference section (214 references taking up 15 pages), the description of tattoos, the treatment of acquired lesions other than naevi, all could have been succinctly précised or left out. Their presence detracts from the laser sections which are uniformly good.

R.P.R.DAWBER

Vasculitis. Edited by KLAUS WOLFF and RICHARD K. WINKELMANN (1980). London: Lloyd-Luke. Volume 10 in Major Problems in Dermatology, many b & w illus., pp. 338. Price £20.00.

This book derives from an excellent meeting held as the 4th ESDR Clinical Symposium in Innsbruck in February 1979. Although covering some ground similar to Ryan's No. 7 in this series, the approach is somewhat different, the two accounts complementing each other.

The first half considers the pathogenesis of vasculitis in all its forms. Inevitably immune complexes are much in evidence, but we are encouraged to look in much greater detail at the other factors which determine how and why complexes, so commonly found circulating, are able to initiate vascular inflammation; also why the vascultis may be perpetuated long after the complexes are no longer in evidence. With such a multiplicity of factors concerned, it is not surprising that there is a good deal of overlap from patient to patient and from time to time, both in the pathological and in the clinical features.

The clinical variants of vasculitis are each accorded a chapter, so that we come away with a far clearer concept of such bewildering names as Churg-Strauss granuloma, lymphomatoid granulomatosis and livedoid vasculitis, as well as all the better known entities. It is noteworthy that all the considerations of the first half cannot be translated into the second half, but the whole concept of an ESDR symposium like this is to be a stepping-stone and not to provide a definitive text.

Much must have been done to organize this symposium into a coherent tale, justifying the 18 months delay in appearance of the book. The two editors between them have contributed to 13 of the 31 chapters and managed to hold the story together in much the same way as the central promenade theme in Mussorgsky's Pictures at an Exhibition. The final treatment chapter gives many useful guidelines other than a blanket use of corticosteroids but has hardly yet achieved the status of the Great Gate of Kiev.

R.H.CHAMPION

Maladie de Bowen. Erythroplasie Carcinome in Situ. P. JEANDIDIER and J. BEUREY (1980). Paris: Editions Medicales. Pp. 104, 28 full plates with colour. Price F.105.00, carriage charges included.

For the English-speaking reader, a relatively short but exhaustive dissertation on Bowen's disease in French will have limited value. Nevertheless, this little book, for those with an interest in cutaneous neoplasia, has distinct merits.

The contents doubtless comprise the material compiled for a 'these', which is an essential requirement for those desiring specialist qualifications in France. The topic has been detailed comprehensively and represents a monumental study of the subject. However, Bowen's disease is common and there is little related to it which is not well known to practising dermatologists. Some sections including that on the induction of skin malignancy by arsenic are more valuable than others. A complicated sub-classification of morphological types such as is outlined is hardly necessary, whereas a more thorough discussion of the more topical concept of 'multicentric pigmented Bowen's disease' might have been appropriate. Despite this, the text is clear and informative and is complemented by superb clinical and histopathological illustrations in colour. The bibliography is vast and probably all-encompassing, which is inevitably useful; greater selection of the more authoritative references is sometimes desirable but the list given allows the reader to consult the literature on almost any aspect of the disease.

D.M.MACDONALD

A Handbook of Medical Hypnosis. Fourth Edition. GORDON AMBROSE and GEORGE NEWBOLD (1980). London: Bailliere Tindall PP. 213. Price £8.50.

This book, written by two medically qualified men, totally lacks any scientific objectivity and reveals an apparent total ignorance of the progress in the methodology and control of therapeutic assessment developed in this century.

It is assumed throughout that any effects of hypnotism are obtained by manipulation of the psyche. The initial chapter on the history and legal aspects of medical hypnotism are of some interest, but thereafter the authors progress from the hypothetical to the realm of pure imagination in their discussion of hypnotism in various specialties.

The book caught the reviewer in a benign mood, and he will therefore rest content in describing the chapter on 'Hypnosis in Dermatology' as ludicrous. The chapter begins with the uncritical acceptance of a list of sixteen conditions (including all the common dermatoses) as having 'been treated effectively by hypnosis'. The authority for this assertion is that McDowell said so in 1953!

A case report is cited as illustrating the importance of emotional factors in skin disease when the death of a father produces dermatological mayhem in his household. 'The younger daughter of 16 was seen one month after the father's death complaining of a weepy irritated rash where her suspender had rubbed her thigh.' Need one go on!

HARVEY BAKER

British Journal of Dermatology (1981) **104**, 358.

Obituaries

Dr D. M. Pillsbury

Dr D.M.Pillsbury of Philadelphia died on 9 October 1980. He had been an Honorary Foreign Member of the British Association of Dermatologists since 1945.

Dr Harold Blum

Dr Harold Blum died in Philadelphia on 29 September 1980. He will be remembered for his substantial contributions to photobiology in the skin.

British Journal of Dermatology (1981) **104**, 359.

News and Notices

Dermatopathology Workshop

A two day Dermatopathology Workshop will be held on 18 and 19 May 1981 at the University of Massachusetts Medical School, Worcester, Massachusetts.

The workshop will include the study of 100 unknown microscopic slides of inflammatory dermatoses and skin tumours. The faculty members include Drs Jag Bhawan, Martin H. Brownstein, Herman Pinkus and Kurt Stenn.

The course is being sponsored by the Office of Continuing Medical Education, Department of Pathology, University of Massachusetts Medical School, Worcester, Massachusetts and co-sponsored by the American Society of Dermatopathology and endorsed by American Society of Clinical Pathology. For further information contact, Jag Bhawan, M.D. (Course-Director), U-Mass Medical School, Worcester, Massachusetts 01605 (U.S.A.), 617 (856-2412).

The First Pan African Congress of Dermatology

This Congress will take place at the Arusha International Conference Centre, Tanzania, from 4 to 10 October 1981. It is open to doctors, health workers, field workers, educationalists and research workers in Africa and other parts of the world interested and/or working in the fields of dermatology, venereology, leprology and allergology/clinical immunology. A registration fee of a sum to be determined later will be required.

The Congress will consist of a scientific programme including recent advances, various symposia, workshops and free communications on the various aspects of dermatological sciences. There will be facilities for exhibits by pharmaceutical, publishing and medical manufacturing companies on payment of a prescribed fee. Other activities will include social events, tours to interesting National Parks in Tanzania and spouse hospitality.

For further information contact: Professor Aaron E.J.Masawe, Chairman, Organizing Committee. The First Pan African Congress of Dermatology, P.O. Box 65001, Dar es Salaam, Tanzania.

National Eczema Society

The National Eczema Society has recently awarded two grants for research. This makes a total of four grants allocated in the first year of operation of the Society's research awards scheme.

An award of £1,959 has been made to Dr C.F.H.Vickers, Consultant Dermatologist at Liverpool University to enable the purchase of micro-computer equipment. This will assist with the storing of information from a long-term study of the natural history of infantile eczema.

A second grant of £2,000 is going to Dr B.J.Levinsky, Consultant Immunologist at the Institute of child Health in London for work on the Characterization of Lymphocyte sub-populations and their relationship to abnormalities in suppressor cell function in children with atopic eczema.

Previous grants this year went to Glasgow University and Westminster Hospital Medical School. The National Eczema Society offers grants to a maximum of £3,000 for research work related to all types of eczema and all aspects of the condition, but is especially interested in topics related to management of the disease.

Further particulars about research awards and instructions on how to apply, as well as other information about the Society, may be obtained from the General Society, The National Eczema Society, 5 Tavistock Place, London WC1H 9SR. The next submission date for grant applications is 14 September 1981.

Mr and Mrs J.N.Taub International Memorial Award

The Department of Dermatology, Baylor College of Medicine, is pleased to announce that the 1980 winner of the Mr and Mrs J.N.Taub International Memorial Award for Psoriasis Research is Irwin M. Braverman, M.D., Professor of Dermatology, Yale University School of Medicine.

Dr Braverman's major scientific achievements are related to the microcirculation of human skin. He has developed ultrastructural criteria by which the various segments of the microcirculation can be identified on morphological grounds. This work has since been confirmed by others. These studies, based on reconstructions of serial sections, permit objective analysis of the microcirculation in both normal and pathological states. He has applied these criteria to the study of psoriasis and shown that psoriatic skin lesions improve in response to therapy with ultraviolet light and tar and to photochemotherapy (PUVA) following alterations in the microvasculature. Through autoradiographic studies he has shown that the primary lesion in psoriasis appears to reside within the epidermis, but that the microvasculature is a modulating influence without whose participation lesions cannot develop.

APPOINTMENTS

University of Edinburgh

John A.A.Hunter, Consultant Dermatologist Lothian Health Board, and Senior Lecturer, Department of Dermatology, University of Edinburgh, has been appointed by the University Court to the Grant Chair of Dermatology at the University of Edinburgh.

Dr Hunter was born in Edinburgh, was educated at Loretto School, Pembroke College, Cambridge, and the University of Edinburgh. During his undergraduate career he played hockey and tennis for Edinburgh University, gaining three hockey blues and representing Scotland at hockey in 1962. He graduated BA in 1960 and MBChB in 1963. After admission to membership of the Royal College of Physicians of Edinburgh in 1967 he graduated MD University of Edinburgh in 1977 and was awarded a gold medal for his thesis entitled 'An ultrastructural study of human epidermal dendritic cells under physical stress and after malignant change.' He was admitted to the Fellowship of the Royal College of Physicians of Edinburgh in 1978.

Dr Hunter spent periods as a research fellow at the Institute of Dermatology in London and at the Department of Dermatology, University of Minnesota. He was appointed lecturer, Department of Dermatology, University of Edinburgh in 1970 and to his present post in 1974. Dr Hunter spent short periods as visiting professor at McGill University, Montreal in 1975 and at the All India Institute of Medical Sciences, New Delhi in February 1980.

His main research interests concern the pigmentary system of the skin and electron microscopy of skin disorders. He has published widely on pigmentary disorders, malignant and other photo-sensitive skin conditions. Dr Hunter has served on the Dermatologists Sub-committee of the Central Committee for Hospital Medical Services and on the Executive Committee of the British Association of Dermatologists and its Investigative Group. He is a founder member of the Scottish Melanoma Group and is at present Secretary of the Scottish Dermatological Society.

Welsh National School of Medicine (University of Wales)

Dr Ronald Marks, Reader in Dermatology in the Department of Medicine of the Welsh National School of Medicine, has been awarded a Personal Chair within the Department. Dr Marks gained an Honours Degree in Physiology from the University of London in 1956 and went on to achieve Honours M.B., B.S. from the same University in 1959. His subsequent professional distinctions include D.T.M. and H. (England) 1961, M.R.C.P. (London) 1965, F.R.C.P. (London) 1977 and M.R.C.Path. 1980.

Dr Marks was appointed to a Senior Lectureship at the Welsh National School of Medicine in 1973, having previously served as a Specialist in Dermatology in the Army, and Consultant Dermatologist at St John's Hospital for Diseases of the Skin, London. In the Autumn of 1976, he was Visiting Professor at Johns Hopkins University, Baltimore, U.S.A. and in 1977 he was appointed to a Readership in Dermatology in the Welsh National School of Medicine.

His major research interests have been in the field of epidermal biology and the pathogenesis of epidermal diseases. He has published numerous books, articles and papers and is an Assistant Editor of the British Journal of Dermatology and Managing Editor of the Archives for Dermatological Research.

In 1981 he will be a Visting Professor at the University of Kiel, thus reinforcing his distinction as a Dermatologist of international standing.

Annual Meeting of the British Association of Dermatologists—London 1981

This will be held in London on 1–4 July 1981 under the Presidency of Dr J. S. Pegum. The business meeting will be held on Wednesday 1 July at 3.30 p.m., and all day Thursday and Friday morning will be devoted to scientific papers. The clinical meeting will be held on Saturday morning, 1 July at the London Hospital. All the other meetings will take place in Kensington Town Hall, Hornton Street, W8. The social and sporting events will be held as usual.

Papers are invited and should be sent in the form of a summary of approximately 200 words (six copies) to the Hon. Secretary, Dr G. M. Levene, The British Association of Dermatologists, 7 John Street, London WC1N 2ES, as soon as possible. If there is more than one author please underline the name of the presenter.

Scientific exhibits are welcomed. Six copies of a title and brief outline should be sent in the first instance to the Local Secretary, Dr Harvey Baker, Skin Department, The London Hospital, Whitechapel, London E1 1BB (envelopes should be marked B.A.D. Scientific Exhibits), as soon as possible.

Single room hostel accommodation is available at Imperial College, South Side, Prince's Gardens, London SW7. The hostel is about 20 minutes walk from Kensington Town Hall, and bed and breakfast will cost around £12 per person per night. Hostel accommodation will be bookable on the B.A.D. Registration Form.

Hotel accommodation is available at the Kensington Close Hotel, Wrights Lane, W8, a few minutes walk from Kensington Town Hall, at rates of approximately £22 per night single room, and £31 per night double room, including continental breakfast. Members must make their own reservations at the Kensington Close Hotel and they are advised to book early.

In considering accommodation members are requested to keep in mind the requirements of their Registrars and Assistants.

Further information may be obtained from the Honorary Secretary, and Dr Baker, the local secretary for the meeting, will be pleased to answer any queries concerning the meeting itself.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr R.H.Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldly names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm³	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 104, Number 3, March 1981

CONTENTS PAGE

Clinical and Laboratory Investigations

The kinetics of metaphase arrest in human psoriatic epidermis: an examination of optimal experimental conditions for determining the birth rate — 231
IAN RALFS, RODNEY DAWBER, TERENCE RYAN, MARK DUFFILL AND NICHOLAS A. WRIGHT

An attempt to use vincristine and colcemid to measure proliferative rates in normal human epidermis in vivo — 243
R.S.CAMPLEJOHN, S.GELFANT AND DAN CHALKER

Pityriasis rubra pilaris: epidermal cell kinetics — 249
I.G.RALFS, R.P.R.DAWBER, T.J.RYAN AND N.A.WRIGHT

Pityriasis rubra pilaris and retinol-binding protein — 253
ALDO F.FINZI, GIANFRANCO ALTOMARE, LUIGI BERGAMASCHINI AND ANTONIA TUCCI

The relevance of morphometry in the differential diagnosis of cutaneous T cell lymphomas — 257
E.M. VAN DER LOO, W.A. VAN VLOTEN, C.J.CORNELISSE, E. SCHEFFER AND C.J.L.M.MEIJER

Flow cytometry as a tool for the study of cell kinetics in skin 2. Cell kinetic data in psoriasis — 271
F.W.BAUER, N.H.C.N.CROMBAG, J.B.M.BOEZEMAN AND R.M. DE GROOD

Autoradiographic investigations on the cell kinetics of epidermis and periderm of limb buds from mouse embryos *in vitro* — 277
R.HERKEN AND U.SCHULTZ-EHRENBURG

Factitious urticaria: Red Dermographism — 285
ROBERT P.WARIN

Further evidence for increased light sensitivity in patients with malignant melanoma — 289
HARRY BEITNER, ULRIK RINGBORG, GORAN WENNERSTEN AND BENGT LAGERLOF

Eye protection in psoralen photochemotherapy — 295
J.B.DAVEY, B.L.DIFFEY AND J.A.MILLER

Cyclic GMP metabolism in psoriasis: increased activity of soluble epidermal cyclic GMP phosphodiesterase and its modulation by calcium — 301
JOHN S.CANTIERI, GUSTAV GRAFF AND NELSON D.GOLDBERG

Epidemiology of congenital pigmented naevi: 1. Incidence rates and relative frequencies — 307
EDUARDO E.CASTILLA, MARIA DA GRACA DUTRA AND IEDA M.ORIOLI-PARREIRAS

HLA antigens in patients with scabies — 317
EDVARD S.FALK AND ERIK THORSBY

HLA-DR3 in dermatitis herpetiformis — 321
HUBERT PEHAMBERGER, KARL HOLUBAR AND WOLFGANG R.MAYR

Pharmacology and Treatment

A clinical evaluation of the effects of an aromatic retinoid (Tigason), combination of retinoid and PUVA, and PUVA alone in severe psoriasis — 325
J.LAUHARANTA, T.JUVAKOSKI AND A.LASSUS

Case Report

Primary lymphoblastic non-Hodgkin's lymphoma of the skin — 333
R.WILLEMZE, W.A.VAN VLOTEN, E.M.VAN DER LOO AND C.J.L.M.MEYER

Brief Communication

Alopecia areata regrowth induced by *Primula obconica* — 339
E.J.RHODES, W.DOLMAN, C.KENNEDY AND R.R.TAYLOR

Comment

Changing concepts in melanoma management — 341
F.RAMPEN

Correspondence — 349

Book Reviews — 355

Obituaries — 358

News and Notices — 359

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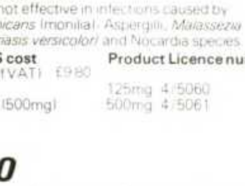

THE INSIDE STORY ON FUNGAL INFECTION

Prescribing information

Dosage and administration

Doses should be taken after meals for maximum absorption.

Adults: normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily.

Children: usually 10mg/kg (5mg/lb) bodyweight daily in divided doses.

Duration of treatment: for hair or skin at least four weeks' treatment is required, whereas finger or toe nails may need six to twelve months' treatment. Continue for at least two weeks after all signs of infection have disappeared.

Contra-indications

Porphyria or severe liver disease.

Griseofulvin may cause deterioration in some patients with liver disease and liver function should be monitored in such conditions.

Precautions

Griseofulvin may decrease the effect of coumarin anticoagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established, but use during pregnancy is best avoided.

Side effects

Headache, and gastric discomfort sometimes occur, but usually disappear as treatment continues. Rarely, urticarial reactions and erythematous rashes have been reported.

Further information

Grisovin is not effective in infections caused by *Candida albicans* (monilia), Aspergilli, *Malassezia furfur (Pityriasis versicolor)* and Nocardia species.

Basic NHS cost		Product Licence numbers
(exclusive of VAT)	£9.80	
Bottle of 100 tablets (500mg)		125mg 4/5060 500mg 4/5061

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PUVA facilities for the treatment of psoriasis are now available to private patients who are referred by Consultant Dermatologists. The Waldmann PUVA 6000 unit, widely acknowledged as the best of its kind, is used. It allows wholebody treatment in the standing position. There are also Waldmann PUVA 180, 200 and 500 units for the treatment of hands, feet and other small areas.

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An Honorary Medical Advisory Committee made up of four consultant dermatologists is supervising the professional standards and practice of the clinic. Dermatologists, who would like to know more about this facility, are asked in the first instance to contact:

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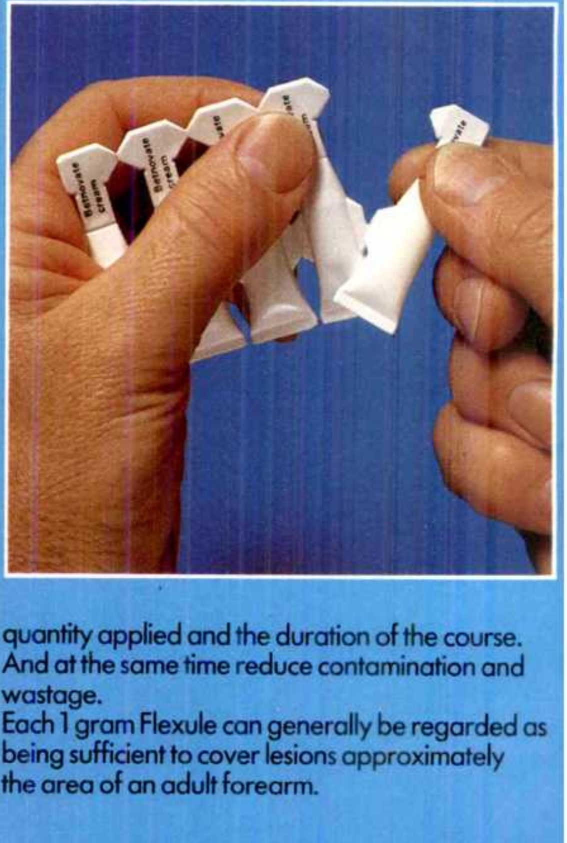

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Prescribing information

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Side effects
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Precautions
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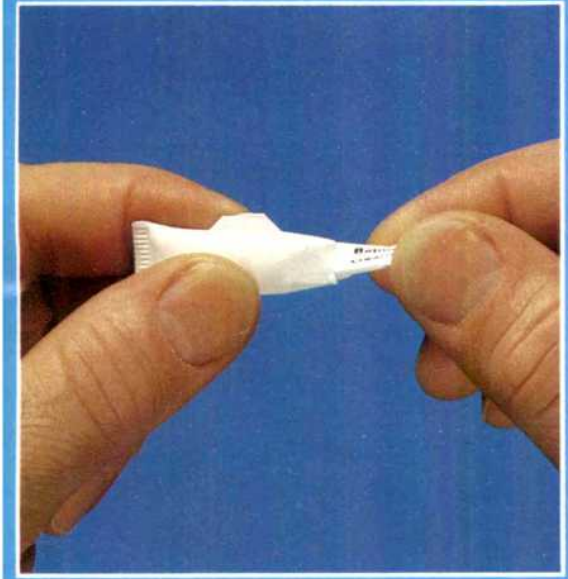

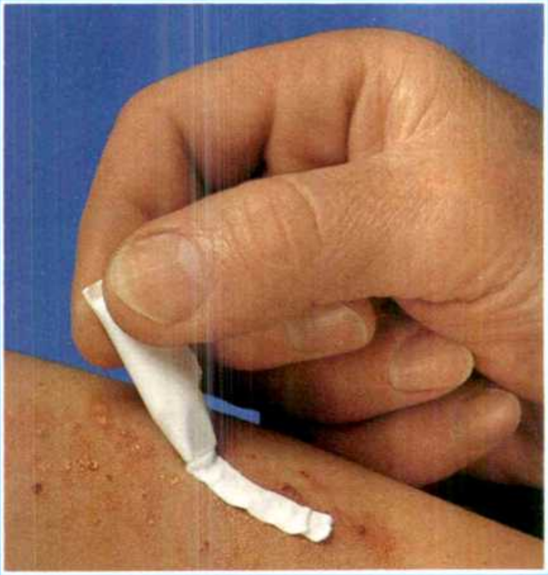

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Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications

Bacterial, fungal or viral diseases of the skin. Dermovate and Betnovate are also contra-indicated for rosacea, acne and peri-oral dermatitis. Hypersensitivity to the preparation.

Basic NHS cost (exclusive of VAT)	**Product Licence numbers**
Betnovate Cream/Ointment Flexules 20 × 1 gram £3·62	4/5121, 4/5124
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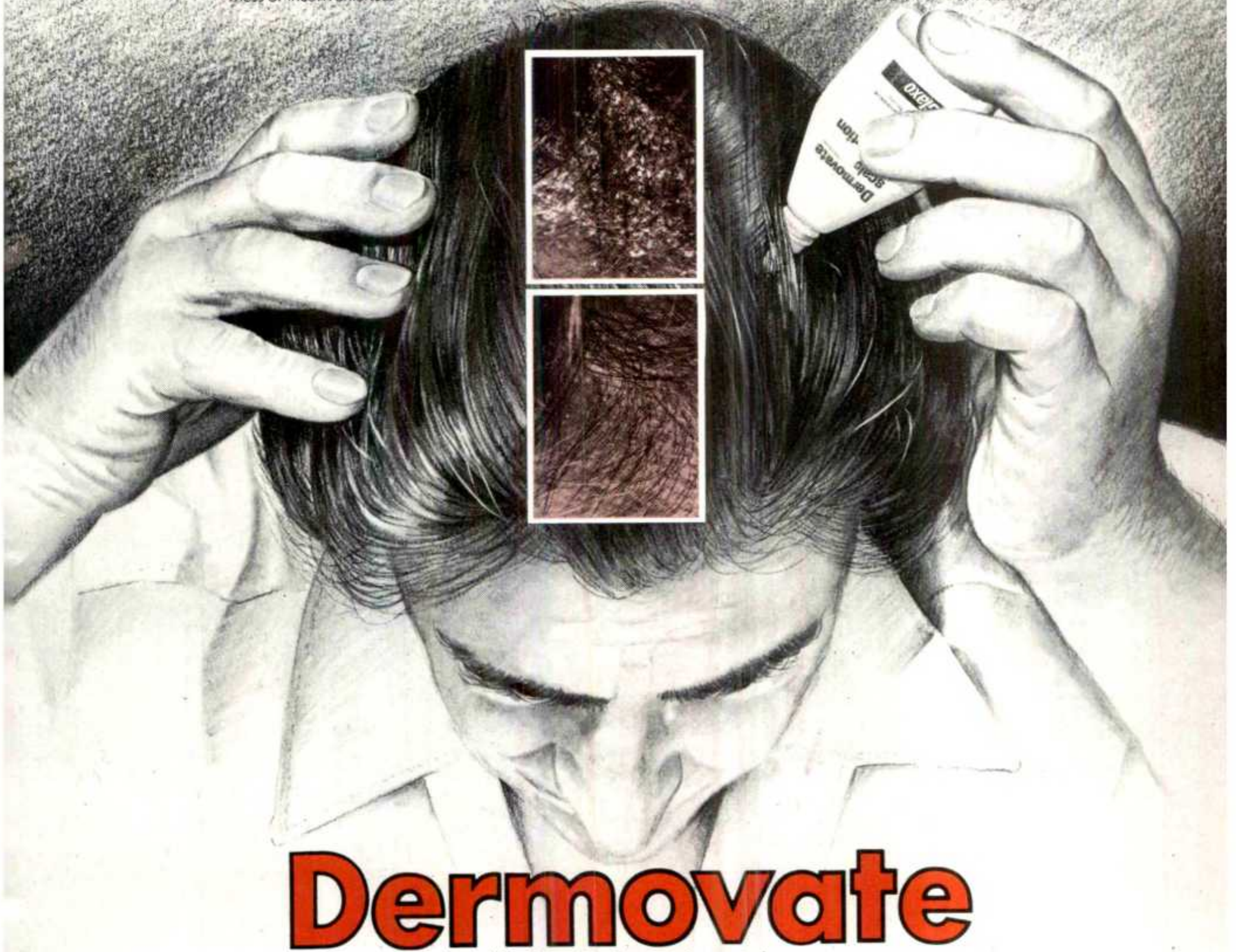

Dermovate

(clobetasol propionate)

Scalp Application

Prescribing information

Uses

Psoriasis and recalcitrant eczemas of the scalp. Clobetasol propionate is a highly-active topical corticosteroid which is indicated for use in short courses for conditions which do not respond satisfactorily to less active steroids.

Dosage and administration

Apply sparingly to the scalp night and morning until improvement occurs. As with other highly-active topical steroid preparations, therapy should be discontinued when control is achieved. Repeated short courses of Dermovate Scalp Application may be used to control exacerbations. If continuous steroid treatment is necessary, a less potent preparation should be used.

Contra-indications

Infections of the scalp. Hypersensitivity to the preparation.

Precautions

Care must be taken to keep the preparation away from the eyes. Do not use near a naked flame. Long-term continuous therapy with Dermovate Scalp Application should be avoided, particularly in infants and children, in whom adrenal suppression occurs readily.

Development of secondary infection requires withdrawal of topical corticosteroid therapy and institution of appropriate systemic antimicrobial therapy.

Topical administration of corticosteroids to pregnant animals can cause abnormalities of fetal development.

In general, topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

Dermovate preparations are usually well tolerated, but if signs of hypersensitivity appear, application should be stopped immediately. As with other topical corticosteroids, local atrophy may result from prolonged treatment, particularly if occlusive dressings are used. When extensive areas are treated, sufficient systemic absorption may occur to produce the features of hypercorticism, especially in infants and children. This effect is more likely to occur after prolonged treatment, particularly if occlusive dressings are used. In rare instances, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Presentation

Plastic squeeze bottle with elongated nozzle containing 25 or 100ml

Basic NHS cost (**exclusive of VAT**)

25ml bottle £2·72

100ml bottle £9·65

Product Licence number

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Leaders in topical steroid therapy

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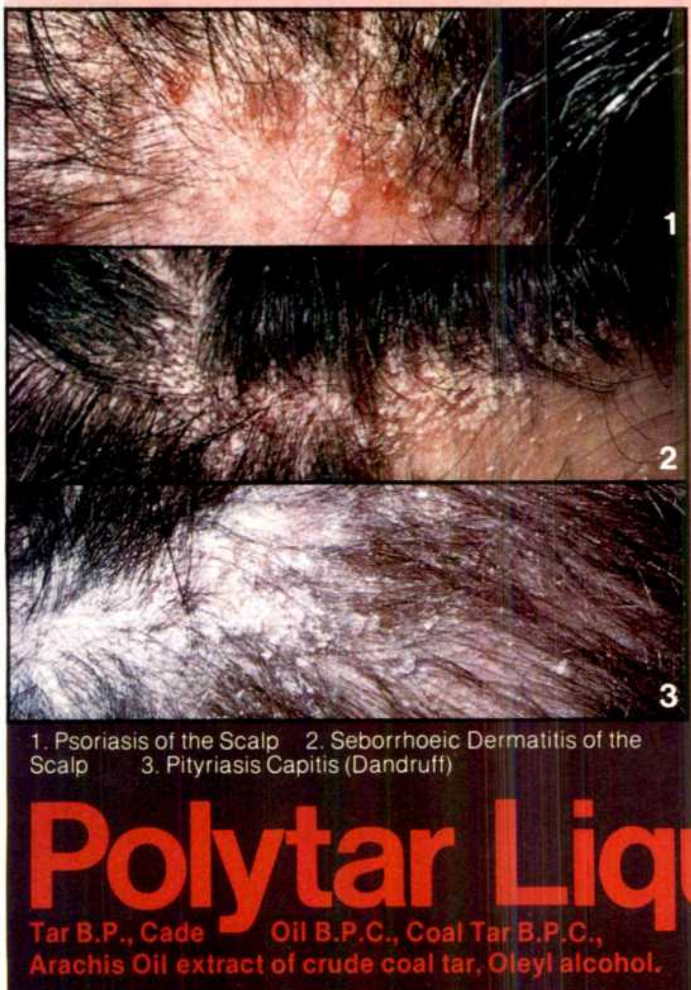
IDEODIS

Common Disorders of the Scalp.

They all have one thing in common — the treatment



1. Psoriasis of the Scalp 2. Seborrhoeic Dermatitis of the Scalp 3. Pityriasis Capitis (Dandruff)

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Prescribing Information.

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Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.

Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
Polytar Liquid	65ml	57p
	150ml	82p
	350ml	£1.48
	1 litre	£3.90
Polytar Plus	150ml	90p

Product Licence Number
Polytar Liquid PL0174/5016 Polytar Plus PL0174/0037

Full information and Data sheet on request.

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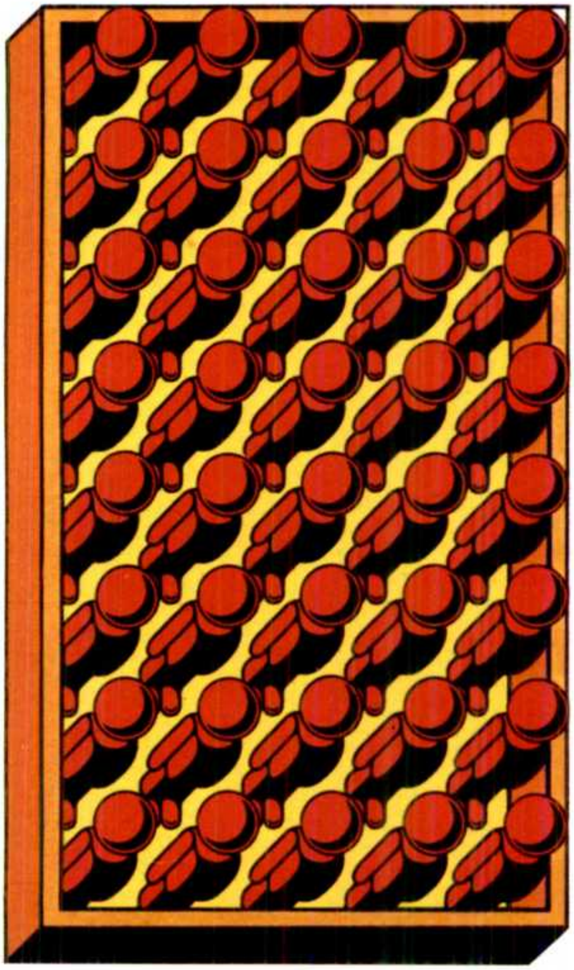
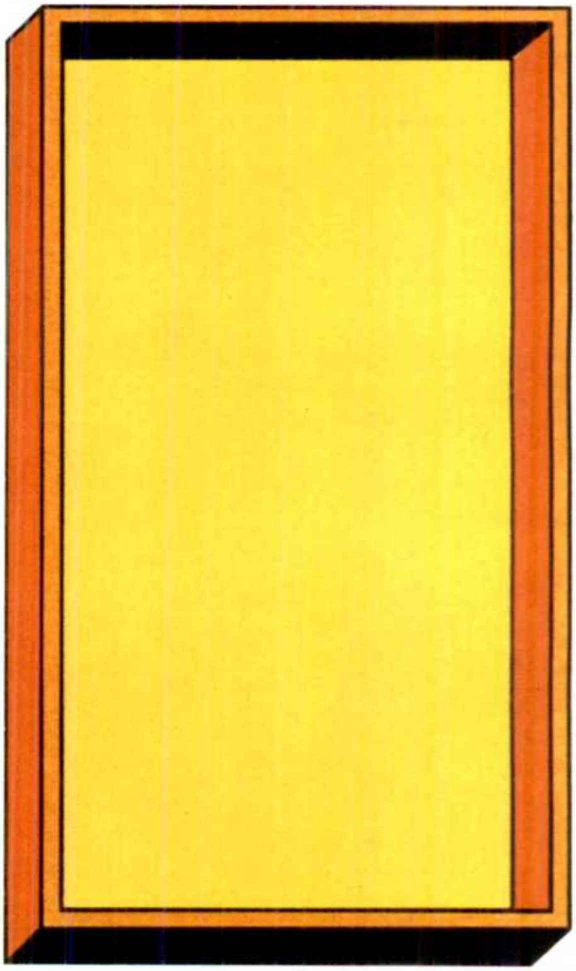


All 40 patients in the trial preferred Dithrocream



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In a recent clinical trial,[1] Dithrocream 0.25% was compared with dithranol ointment of equivalent strength in the treatment of psoriasis.

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Indications

For the topical treatment of subacute and chronic psoriasis including psoriasis of the scalp. Dithrocream Forte should only be used for resistant cases.

Directions for use

Apply sparingly to the psoriatic lesions preferably at night and rub in gently and carefully until absorbed. Bathe before the next application. For scalp lesions, shampoo before use. Continue treatment until the skin is smooth and clear.

Contraindications

Acute psoriasis; dithranol sensitivity.

Precautions

Keep away from the eyes. Apply to psoriatic areas only. Use cautiously after recent application of potent steroids. Wash the hands after use.

Basic N.H.S. prices:

Dithrocream 0.1% 50gm tubes £2.10
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Dithrocream Forte 50gm tubes £2.54

Product Licence Nos.

Dithrocream 0.1%	PL 0173/0029
Dithrocream 0.25%	PL 0173/0028
Dithrocream Forte (0.5%)	PL 0173/0027

References:

1. *British Journal of Dermatology*, 1979, **100**, 475

Full prescribing information is available from
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Now available in three strengths

British Journal of Dermatology (1981) **104,** 369.

Clinical and Laboratory Investigations

Recurrent urticaria: clinical investigation of 330 patients

LENNART JUHLIN

Department of Dermatology, University Hospital, S-750 14 Uppsala 14, Sweden

Accepted for publication 23 May 1980

SUMMARY

A questionnaire was used from 1972–1978 for 330 consecutive patients with recurrent urticaria of 3 months to 40 years duration. Fifty men and fifty women had only urticaria. The rest had both urticaria and angio-oedema and most of them were women between 24 and 38 years of age. Urticarial attacks were less frequent during the daytime. A personal history of rhinitis, asthma or atopic dermatitis was recorded in more than one-third. Nasal polyps, migraine and arthralgia were found in 6–7% of the patients. Severe psychiatric problems were mentioned by 16%. Abdominal problems, mainly gastritis, were described by 44%. A history of side effects from drugs was found in 32% of the patients. Food was mentioned as a factor worsening the weals by 30% and drinks by 18%. Fruits, vegetables and nuts were the most common. Despite all cases with physical urticaria having been excluded, physical factors such as exercise were considered by 20% to make the urticaria worse. Provocation tests with various food additives such as azo dyes, benzoates, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) sorbic acid, quinoline yellow, carotene, canthaxanthine, annatto and nitrite revealed one or more positive reactions in one-third of the patients, one-third showed negative provocation tests and, in the rest, one or several tests were questionable. Routine laboratory investigations and X-rays of sinuses or teeth were of little value if there was no sign or history of other diseases. The fibrin microclot generation test which indicates the presence of circulating endotoxins was positive in 24%.

Recurrent urticaria is always a challenge to the dermatologist. Sometimes one can find a specific agent which causes the patient's urticaria but it is often time consuming and needs an observant patient and a doctor with a mind of Sherlock Holmes. The doctors have used a questionnaire from 1972–1978 for 330 consecutive patients with recurrent urticaria. The intention was to investigate what might be most relevant in the case history and which investigations and tests are worth doing.

Definitions

All patients had had repeated attacks of urticaria for more than 3 months. In some patients the periods without lesions were longer than those of wealing, i.e., 'recidivating' urticaria and, in others,

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the period with weals was longer, i.e., chronic urticaria. Since a strict differentiation between the two types of urticaria is often difficult, I have here used the term recurrent urticaria. Patients with any type of physical urticaria or hereditary angioneurotic oedema have been excluded, as well as fifteen patients with pure angio-oedema.

Age of patients, duration, frequency and diurnal variation of urticaria

A total of 121 men and 209 women with chronic urticaria were investigated on the wards from 1972–1978. Fifty men and fifty women had only urticaria. The rest also had attacks of angio-oedema. Most of the women were between the ages of 24 and 38 (Fig. 1). The age distribution in men showed no such peak and most were between 18 and 58 years of age (Fig. 2). The patients had had urticaria for more than 3 months up to 40 years (Fig. 3). Most of them had had urticaria daily or a couple of times a week (Table 1).

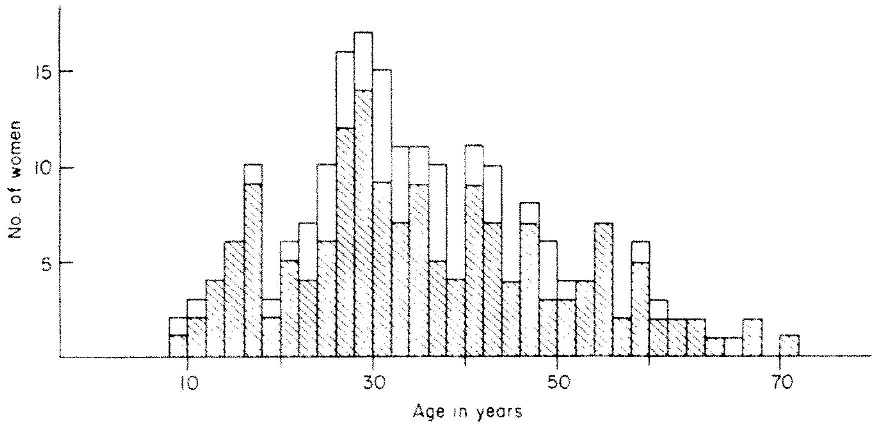

FIGURE 1. Age distribution of females with urticaria. □ Urticaria; ▨ urticaria + angio-oedema.

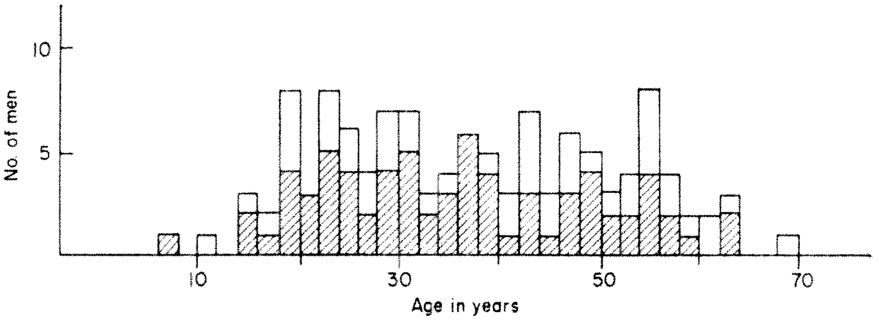

FIGURE 2. Age distribution of males with urticaria. □ Urticaria; ▨ urticaria + angio-oedema.

Seasonal variation was noted in 25% of the patients, but the period when the urticaria worsened was evenly distributed over the year. Diurnal variation was mentioned by 53%. Urticaria occurred less frequently during the daytime (Table 2). Of the thirteen patients with urticaria mainly during the day, four were found to be sensitive to chemicals at work and four complained of heat and stress at work.

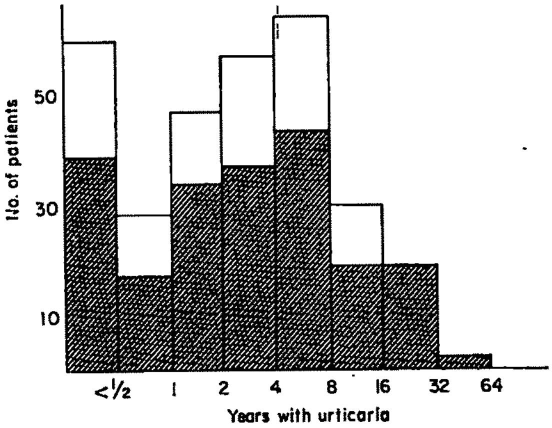

FIGURE 3. Duration of urticaria. ☐ Urticaria; ■ urticaria+angio-oedema.

TABLE 1. Frequency of urticarial eruptions

	Percentage
Daily	44
Some times per week	24
Some times per month	18
< 1 time per month	14

TABLE 2. Diurnal variation found by 176 of 330 patients (53%). Two periods mentioned by thirty-eight patients

Time (h)		Percentage
Morning	6–8	23
Daytime	8–18	7
Evening	18–23	44
Night	23–6	26

Additional diagnosis

The patients had or had been treated for additional disorders which are listed in Table 3. Rhinitis and asthma were common and severe depression or psychiatric problems were mentioned by 16%. Of special interest is that 44% had asked advice for abdominal troubles at some time during the previous 5 years. The most common diagnoses were gastritis, diarrhoea and abdominal swelling (Table 4). The symptoms were usually linked to the period with urticaria.

The attacks of angio-oedema were less frequent than those of urticaria. The sites mentioned by the 230 patients are listed in Table 5 in percentages since many had swellings of more than one area.

TABLE 3. Recurrent urticaria 330 patients

Additional diagnosis	Percentage
Angio-oedema	70
Rhinitis	20
Asthma	11
Atopic dermatitis	9
Nasal polyps	7
Migraine	7
Arthralgia	6
Thyroid disease	4
Purpura	3
Contact dermatitis	3
Depression, psychiatric problems	16
Abdominal trouble	44
Various disorders	18

TABLE 4. Abdominal trouble mentioned by 44% of the patients with recurrent urticaria

Type of diagnosis	Percentage of patients with abdominal trouble
Gastritis	50
Attacks of diarrhoea	20
Abdominal swelling	10
Constipation	7
Cholecystitis	5
Various	8

TABLE 5. Localization of angio-oedema

	Percentage
Lips	22
Eyelids	18
Face	16
Tongue	10
Throat, larynx	8
Ears	2
Hands, feet	11
Around knee	7
Abdomen	3
Penis, scrotum	2
Whole body swollen	1

Infections

Investigations for foci of infection were done as soon as there was any suspicion. X-ray investigation for dental foci was done in seventy-eight patients with a poor dental status or a history of dental symptoms. A possible dental focus was found in 6% and a sinus focus in 4% of all the patients. In ten patients the dental focus was treated by tooth extraction. The urticaria disappeared after a week in three of them. Recurrent infections of the urinary tract were found in six patients and recurrent tonsillitis in three. The effect of antibiotics was difficult to evaluate but was not impressive.

In the case histories, 20% were found to have had an appendicectomy, 10% a cholecystectomy, 8% a tonsillectomy and 2% had nephrolithiasis. The percentages do not differ significantly from those seen in the general population.

Intolerance to drugs and foods

Thirty-two per cent of all patients stated that, at some time in their life, they had had untoward reactions to one or more drugs (Table 6). Pencillin was the drug mentioned most commonly. The reactions to penicillin were urticaria and angio-oedema (42%), maculopapular rash (34%), shock (8%), pruritus (5%), vomiting (5%) and various (5%). The aspirin- and indomethacin-intolerant

TABLE 6. Drugs not tolerated by 330 patients with recurrent urticaria

Type of drug	No. of patients	Percentage
Penicillin	38	11
Aspirin	33	10
Sulphonamides	21	6
Antihistamines	9	3
Iron	5	2
Iodide	4	1
Indomethacin	4	1
Tetracycline	4	1
Various others	18	5
Total	136	40

TABLE 7. Type of food believed to provoke urticaria

	No of patients
Fruit, vegetables, nuts	76
Fish, shellfish	25
Ketchup, spices, vanilla	20
Egg	15
Milk products	10
Cheese	10
Bread	5
Fried foods	4

patients had, as a rule, reacted with urticaria and angio-oedema. Antihistamines had caused severe fatigue, irritability or other psychological reactions. The reactions to the other drugs mentioned by the patients varied but a poorly defined skin rash was the most common.

The patients were asked if there were foods, sweets or drinks which they thought might provoke attacks of urticaria. Food was mentioned by 30%, drinks by 18% and chocolate and sweets by 10%. Ready-made products and preserved food were mentioned by 4 and 3% respectively. The types of food and drinks believed to provoke urticaria are shown in Tables 7–8. Fruit and vegetables were most commonly mentioned and especially citrus fruits, apples and strawberries. Nuts were especially mentioned by the atopics.

TABLE 8. Type of drinks not tolerated by fifty-six patients

	No. of patients
Soft drinks	24*
Fruit juices	11*
Wine	16†
Beer	7
Alcoholic drinks	14
Coffee	4

* Orange or coloured drinks specified by seventeen patients.
† Only red wine five, only white wine three patients.

Stress and physical factors

In our material all patients with pure physical urticaria were excluded. Nevertheless, 20% of the patients stated that physical factors such as exercise, heat, pressure and cold increased the number and size of weals and made them worse (Table 9). Seven per cent felt that stress and nervousness made their urticaria worse.

TABLE 9. Number of patients finding that their urticaria gets worse from the following factors

	No. of patients	Percentage of all patients
Exercise, sweating, heat	36	11
Stress or nervousness	22	7
Pressure	21	6
Cold	9	3
Dust	6	2
Fumes from solvents, diesel, paints	5	2
Common cold	3	1
Various: dandruff, feathers, sunshine, dry weather, washing powder	5	2

Seven patients mentioned both heat and pressure and four both heat and nervousness. Three patients mentioned the combinations dust-heat, cold-heat and cold-pressure.

Laboratory abnormalities

Routine tests were done for haemoglobin, WBC, differential count, ESR, serum transaminases, ANF, creatinine, and urinary glucose, protein and sediment. As a test of thyroid function, protein-bound iodine (PBI) was used in the first half of the patients. Later T_3, T_4 or TSH were measured. Serum immunoglobulins, RAST with a standard set of ten antigens (animal dander, pollens, dust, milk, egg and nut), and ANF were done in the first 100 patients. The results are shown in Table 10. Many of the patients were atopics which can explain the increase of eosinophils and IgE, as well as the positive radioallergosorbent tests (RAST). The thyroid function tests were abnormal in 6%. Most of these patients had been operated on for hyperthyroidism or were under medical treatment with thyroid hormones. Of the newly detected cases three had signs of hyperthyroidism and one myxoedema. The ANF titre was slightly raised 1/10–1/40 in 8%.

TABLE 10. Laboratory abnormalities

Test	Percentage
Eosinophilia (>4%)	8
IgE increase	4
RAST	8
Thyroid	6
Antinuclear factor	8
ESR >30 mm/h	2
Urine bacteria	4

Microclot generation (MCG) test

The test was performed as earlier described in detail (Juhlin & Shelley, 1977). Bacterial lipopolysaccharides (*E. coli*) were added to the patients' buffy coat which was then taken up into capillary tubes and left for 24 hours at 37°C. The number of fibrin microclots appearing as stars were counted under a phase interference microscope. The MCG-test with added endotoxin was positive in 56% of the seventy-four patients tested. In 24% it was positive without adding a lipopolysaccharide. We were not able to find any correlation between the MCG-test and the severity of urticaria or any special type of information obtained from the answers of the questionnaire.

Provocation tests

The patients were recommended a diet free from salicylates and dyes for at least 4–5 days prior to admission. No antihistamines were given. For safety reasons and to allow close examination, all the provocations were performed in the wards. A challenge test battery with several controls, as shown in Table 11, was used from 1974–1978. Changes in the schedule were often made. The tests could only be done in patients with no or slight symptoms. The chemicals were given in titanium dioxide-whitened gelatin capsules with lactose added to complete the fillings and the patients were unaware of the type of compound tested. If the patients reacted to lactose, wheat starch was used instead as filling material. The first dose was given at 8 o'clock in the morning and additional doses at 1-h intervals. Only one type of additive was given per day. If the test was positive, it was necessary to wait with the next provocation until the reaction had subsided. Provocation tests are judged as positive when the patients develop clear signs of urticaria or angio-oedema within 24 h. About half

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TABLE 11. Current test battery for patients with recurrent urticaria

Day	Substances	Amount
1	Control (lactose)	100, 100 mg
2	Azo dyes: Tartrazine	0·1*, 1, 10 mg
	New Coccine	0·1*, 1, 10 mg
	Sunset Yellow	0·1*, 1, 10 mg
3	Control (lactose)	100, 100 mg
4	Benzoates: Sodium Benzoate	50, 500 mg
	4-hydroxybenzoic acid	50, 200 mg
5	Carotene	50, 100, 100 mg
	Canthaxanthine	10, 200, 200 mg
6	Annatto	5, 10 mg
7	BHT-BHA	1, 10, 50, 50 mg
8	Yeast extract	0·6 g
9	Control (lactose)	100, 100 mg
10	Aspirin	0·1*, 1, 10, 100, 250†, 500† mg
11	Sorbic acid	50, 200, 200 mg
12	Control	
13	Sodium nitrite	100 mg
	Sodium nitrate	100 mg
14	Quinoline Yellow	1, 5, 10 mg
15	Sodium glutamate	100, 200 mg

* 0·1 mg only in patients with a history of asthma.
† Not to be given if history of asthma and severe aspirin reactions.

of the patients reacted within 6 h. Judging whether a reaction is positive or negative is not always easy. When in doubt, the doctor should therefore call the reaction uncertain and repeat the test later. One or more positive reactions were found in 31% of the patients tested and totally negative tests in 36%. In 33% one or several tests were uncertain. The results of the individual provocation tests appear in Table 12. They are those reported by the doctors on the questionnaire for provocation tests. Some patients reacted to more than one drug. Patients reacting to lactose were tested again with starch. In five patients the repeated test with lactose was positive but the test with starch was negative. The tests with other chemicals were then done with starch as filling. In four other lactose-positive patients, the repeat tests with lactose were negative. Several patients with ongoing urticaria had questionable tests to both lactose and starch. Further tests then had to be postponed until the patient was in a more stable phase. Questionable tests to other chemicals were often repeated. If later positive, the test was then regarded as positive, if negative, it was listed as negative.

Patients with positive or questionable tests were always recommended a diet free from the additives they had reacted to. In patients with positive reactions to additives which have not before been known to provoke urticaria, repeated tests with two placebo capsules were done within a year. The additives tested and the number of patients reacting out of the number provoked were as follows: butylhydroxytoluene (BHT)+butylhydroxyanisol (BHA) 3 of 8, sorbic acid 2 of 5, canthaxanthine 3 of 3, quinoline yellow 2 of 3 and nitrate and nitrite 1 of 2. The patients had often found flare-ups of their weals when they had ingested by mistake the additive they should have avoided.

TABLE 12. Provocation tests in patients with recurrent urticaria. Test period 1974–1978

Substance tested	Positive	Percentage Uncertain	Negative	No of patients tested
Lactose	5	16	79	191
Azo dyes (see table 11)	18	14	68	179
Benzoates	11	18	71	172
Aspirin	22	14	64	149
Annatto, β-Carotene	10	14	76	112
BHT, BHA	15	12	73	156
Sodium glutamate	4	12	81	72
Sodium nitrite, nitrate	6	12	82	60
Sorbic acid	9	14	77	115
Quinoline Yellow	13	15	72	91
Canthaxanthine	14	24	62	42
Yeast	16	14	70	57

DISCUSSION

Occurrence

Recurrent urticaria is more common in women than in men. In our material there was no sex difference in the group of patients having only urticaria, whereas there were more than twice as many women than men having both urticaria and angio-oedema. The patients are usually reported to be between 20 and 50 years of age (Urbach & Gottlieb, 1946; Miller *et al.*, 1968; Champion *et al.*, 1969; Rives *et al.*, 1972; Meynadier *et al.*, 1979). Most of our female patients were between 24 and 44 years of age while the men were more evenly distributed from 12 to 64 years of age (Figs 1–2).

Many of our patients had severe urticaria, with daily eruptions occurring in 44 patients. The median duration of attacks was between 2 and 4 years and the longest duration occurred among those with both urticaria and angio-oedema, which was also observed by Champion *et al.* (1969). Seasonal variation was mentioned by 25% and diurnal variation by 53% which is similar to the findings of Miller *et al.* (1968). Interestingly, only 7% had their urticaria between 8 a.m. and 6 p.m., which means that here the lesions are rarely seen by the doctor unless the patient is in the hospital. In the few patients with urticaria mainly during daytime, chemicals and stress factors at work are worth looking for as was also pointed out by Urbach & Gottlieb (1946).

Angio-oedema is most common in the lips, eyelids, face and tongue as observed by Champion *et al.* (1969). In our material, it was also quite common. In other areas such as the hands, feet, around the knees, abdomen, genitalia and the whole body, it was not spontaneously mentioned if not directly asked for.

Other disorders

Champion *et al.* (1969) found a previous history of atopic disorders in 18·5% of their 438 patients with urticaria, which was not significantly more than the 12·5% they found in 200 controls. These findings were confirmed by Douglas (1975) but when he used a definition of atopy involving both a history of atopy and positive intracutaneous tests, he found a significantly increased number of atopics (27%) in idiopathic urticaria. Meynadier *et al.* (1979) obtained a personal history of 24% atopic diseases in chronic urticaria. Rhinitis was most common and seen in 15%. Miller *et al.* (1968) found rhinitis in twenty-seven of the fifty patients investigated.

In our material 20% of the patients had a personal history of vasomotor rhinitis which is more than in a normal population. The rhinitis was combined with nasal polyps in one-third of the cases and most of them showed intolerance to aspirin. Urticaria is known to be a common symptom in aspirin-intolerant patients with rhinitis and also in some with asthma (Settipane & Pudupakkam, 1975). Such a rhinitis is mostly not of an allergic type and should not be included in the group of atopic symptoms. Thune & Granholt (1975) found atopic disease in eight of their 100 patients with recurrent urticaria. It is close to our figure with a personal history of 9% with atopic dermatitis which suggests a connection with urticaria. The eosinophilia, increased IgE and positive RAST observed were mainly confined to these patients.

A personal history of migraine was obtained in 7%. That migraine can be associated with urticaria in female patients was pointed out by Urbach (1937). Arthralgia was noted in 6% of our patients. An elevated ESR was found in five of them. No biopsy was taken to exclude necrotizing venulitis which has been described in such patients together with abdominal pain (Soter, 1977). In the material studied by Miller *et al.* (1968) 34% complained of arthralgia which in half of them was related to the attacks of urticaria. Thyroid disease was found in 6% of our patients and in 4% of those investigated by Thune and Granholt (1975). Most of them had been treated before the urticaria appeared and were being treated with thyroid hormones. By decreasing the dose, the urticaria often improved and completely disappeared in two patients. That a control of the hyperthyroid state is of importance for the appearance of weals has been mentioned by several authors (Urbach & Gottlieb, 1946; Isaacs & Ertel, 1971).

There was a history of psychiatric treatment for depression or other psychiatric problems in 16% of the patients. Most of them were women. In many of our cases there was also a clear relationship between the attacks of urticaria and psychological factors as mentioned in the literature (Rees, 1957; Warin & Champion, 1974). In the study of Champion *et al.* (1969) psychological factors were considered to be the main cause in 12% and a contributing factor in an additional 24%.

Gastrointestinal symptoms were mentioned by 44% of our patients. Others have reported 38–60% (Lindemayer, 1954; Schmidl & Kern, 1964; Miller *et al.*, 1968; Meynadier *et al.*, 1979). Gastritis was the most common diagnosis. Since it often occurs simultaneously with urticarial attacks, it is feasible that it could be caused by histaminaemia or by an extracutaneous release of histamine. Some authors have described a decreased hydrochloric acid excretion after injection of histamine in patients with chronic urticaria (Schmidl & Kern, 1964; Gloor *et al.*, 1972; Szarmach *et al.*, 1972). Patriarca *et al.* (1978), however, found either an increased or a decreased response in half of their patients. They also observed functional modifications of the X-ray picture in 70% of their 145 patients with chronic urticaria. Endoscopic changes were seen in one-third and histological examinations revealed changes of the gastro-duodenal mucosa in 75%. Peker and Goetzki (1969) reported a decreased proteolytic activity of the duodenal juice and suggested that a reabsorption of non-digested proteins could be a contributing factor in chronic urticaria. Schmidl and Kearn (1964), however, found no gastrointestinal disturbances in their material, whereas Gloor *et al.* (1972) demonstrated a shorter stomach evacuation time in a group of forty-five patients with chronic urticaria. That gall bladder disease is common in patients with chronic urticaria was found by Urbach (1937), Michaëlsson (1969) and Meynadier *et al.* (1979). Lindemayer (1954) found cholecystitis in 14% of his cases. Michaëlsson (1969) observed that a cholecystectomy had been performed in ten out of nineteen women above 40 years of age. In the present material, 10% of the patients had had a cholecystectomy. The figure would be higher if we select females over 40 years of age but is not of the same magnitude as reported 10 years ago. The finding of 20% appendicectomies in our patients is what can be expected in a control material (Romanus, 1978).

Worsening factors

Intolerance to one or more drugs was mentioned by 32% of the patients. Penicillin and aspirin dominated. An intolerance to drugs has been reported before in 15–50% of patients with chronic urticaria (Green *et al.*, 1965; Miller *et al.*, 1968). In the patients investigated by Douglas (1975) penicillin was the most common drug. A personal history of aspirin intolerance has been reported in 22–36% (Moore-Robinson & Warin, 1967; Champion *et al.*, 1969; Ros *et al.*, 1976).

Foods are common causes of acute urticaria but, in those with chronic urticaria, Champion *et al.* (1969) found only 2% with food sensitivity. Green *et al.* (1965) found that foods could be a contributing factor in 16% of their patients. Food dislikes and avoidances were mentioned by 58% of the patients studied by Miller *et al.* (1968). Thirty per cent of our patients believed that fruits, vegetables, nuts, fish, spices, eggs and/or milk products provoked or worsened their urticaria. Two patients were RAST positive to nuts and two to fish. Eighteen per cent felt that drinks provoked their urticaria. In some, provocation tests were done. With wine and beer, severe angio-oedema was seen within some hours. Despite avoiding all the products mentioned, however, they still continued to have weals. Positive provocation tests to fruits, vegetables and nuts were found in 8% of the patients tested by Thune and Granholt (1975). They also had positive provocations to seafood, milk and eggs.

That physical factors such as pressure can worsen chronic urticaria and precipitate attacks is well known (Green *et al.*, 1965; Illig, 1969; Warin & Champion, 1974). Cholinergic urticaria has been found to overlap with idiopathic urticaria in 20–30 per cent (Miller *et al.*, 1968; Douglas, 1975). It was less frequent in our material which might be because we have been careful to exclude pure physical urticarias.

Provocation tests

Various food additives were tested in most patients. The results from the patients studied from 1972–1973 have already been published and revealed that intolerance to azo dyes, preservatives and benzoates was a common feature (Michaëlsson & Juhlin, 1973; Ros *et al.*, 1976). At that time the patients with suspected intolerance were referred to us from other skin clinics for testing. The material was therefore quite selected. Since then the picture has changed and most patients and doctors try to eliminate the additives and the clinics mainly refer those patients with whom they still have a problem. This gives us probably another selection of patients with fewer patients sensitive to dyes and preservatives. With the introduction in Sweden of labelling of additives and removal of azo dyes from all food except caviar, cocktail cherries, fruit cocktails and some alcoholic drinks, life has become much easier for the patient. These factors could explain why we now see fewer patients with azo dye, benzoate and aspirin intolerance than before. Our figures for the incidence of such intolerance reactions are now of the same magnitude as reported by Brodthagen *et al.*, 1974; Thune & Granholt, 1975; Warin & Smith, 1976; Douglas, 1975; Mikkelsen *et al.*, 1978; Meynadier *et al.*, 1979; Wüthrich & Häcki-Herrmann, 1980. Another factor is that the tests now are read by several doctors, many of whom prefer to judge the reaction as uncertain. To evaluate the provocation tests can often be quite difficult unless repeated tests are done. Patients who had reacted to chemicals which previously had not been used in provocation tests were retested within a year. Some of them did not then react again. That they do not react during certain periods has been found before for aspirin, benzoates and azo dyes (Warin & Smith, 1975; Ros *et al.*, 1976). With aspirin it is also clear that a minimum dose is needed to get a reaction. Thus they sometimes react to 50 mg and next time they need 1000 mg to react. The mechanism is still unknown but the blockade of a naturally occurring inhibitor which prevents urticaria and angio-oedema could be a possible explanation.

Most patients improve when given a diet free from the additives to which they had reacted (Ros *et al.*, 1976). This has also become evident in the present investigation in patients reacting to other additives like butylhydroxytoluene (BHT), butylhydroxyanisole (BHA), sorbic acid, nitrite and non-azo dyes such as quinoline yellow and canthaxanthine.

Laboratory investigations

Routine laboratory tests or X-rays of sinuses or teeth are generally of little value in recurrent urticaria. Only if there is a history or sign of another disease are such investigations rewarding. The fibrin microclot formation test when bacterial lipopolysaccharides is added to the patient's blood *in vitro* was positive in 56% of the patients tested. The microclots are never seen in healthy subjects but occur in blood from patients with such disorders as vasculitis, psoriasis, severe eczema and ulcerative colitis (Juhlin & Shelley, 1977). That the test was positive in 24% without adding lipopolysaccharides indicates the presence of circulating endotoxins. It is possible that circulating endotoxins might act as a contributing factor in certain patients with recurrent urticaria by a mechanism similar to the fibrin microclot formation seen *in vitro*.

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Hepatitis B surface antigen deposition in the blood vessel walls of urticarial lesions in acute hepatitis B

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SUMMARY

Urticaria is known to occur in the pre-icteric phase of acute viral hepatitis. Often such urticaria is a symptom of a serum sickness-like syndrome which can be a prodrome of viral hepatitis. Immune complexes are thought to be pathogenetic in this condition.

In this paper we present two patients with a serum sickness-like syndrome, urticaria and hepatitis B. By immunofluorescence techniques Hepatitis B surface antigen, C1q and C3 could be demonstrated in the blood vessels of the superficial dermis. These findings are consistent with the immune complex hypothesis.

INTRODUCTION

In 1843 Graves was the first to describe the occurrence of urticaria in a patient who was suffering from apparent viral hepatitis (Graves 1843).

Immune complexes are thought to be involved in the pathogenesis of the urticarial eruptions in viral hepatitis patients since a serum sickness syndrome with generalized malaise, fever and arthralgia is often present (Duffy *et al.*, 1976; Lockshin & Hurley, 1972; Trepo *et al.*, 1974). Circulating immune complexes of hepatitis B surface antigen (HBsAg) and hepatitis B surface antibodies (HBsAb) have been isolated from the serum of hepatitis patients (Wands *et al.*, 1975). Moreover deposition of a hepatitis B antigen (HBAg) and complement has been demonstrated in the glomerular capillary walls (Combes *et al.*, 1971; Takehoshi *et al.*, 1979), in the synovial fluid (Schumacher & Gall, 1974) and in the liver (Ray *et al.*, 1979).

Recently two studies were reported in the literature in which urticarial and maculopapular lesions of patients suffering from viral hepatitis B were examined for HBsAg deposition (Dienstag *et al.*, 1978; Weiss *et al.*, 1978). Both studies used immunofluorescence techniques. Although Weiss *et al.*, (1978) could demonstrate complement deposition in the skin lesions, they were not able to demon-

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strate HBsAg in the blood vessel walls. In contrast to these observations Dienstag *et al.*, (1978) observed HBsAg-HBsAb complexes as well as IgM in the urticarial lesions.

We studied two patients with urticaria in the prodromal phase of acute viral hepatitis B and also found HBsAg and complement deposition in the small dermal blood vessel walls in the urticarial lesions.

MATERIALS AND METHODS

Patients

Case 1. A 30-year-old homosexual man was admitted to our hospital because of a 5 day history of urticaria. One day before admission the urticaria became generalized and he complained of general malaise, fever, and pain in the hands, wrists and right ankle. He denied any past history of urticaria, allergy or rash. In the past he had been treated for syphilis.

Physical examination showed generalized urticaria and painful joints which later became swollen, red and warm. The individual urticarial lesions disappeared within 24 h. The liver edge was palpable 2 cm below the right costal margin. The rest of the physical examination was normal.

Laboratory studies disclosed the following values: WBC $18{\cdot}5 \times 10^9$/l with differential cell count of 7% band neutrophils, 80% polymorphonuclear leucocytes, 9% lymphocytes and 4% monocytes. Hb: 8·8 mmol/l; ESR 20 mm/h; urinalysis normal. Serum creatinine 78 μmol/l; SGOT 3 U/l; SGPT U/l; serum alkaline phosphatase 35 U/l; γGT 4 U/l; LDH 69 U/l; Total serum bilirubin normal. Serum uric acid normal. AST: 325 U/l (slightly increased). Rose test: negative. ANF: negative. HLA B28: negative. Total serum protein 62·0 g/l; serum albumin 32·7 g/l; α-1: 4·3 g/l; α-2: 10·4 g/l; β 6·3 g/l; γ glob.: 8·1 g/l. C1q: 62% (normal 69–128%) C3: 72% (normal 66–124%); C4: 52% (normal 37–161%); C5: 106% (normal 73–135%). Syphilis serology: TPI negative; FTA-ABS: positive; RPCF, VDRL, Kolmer negative. X-rays of the chest, right hand and knees were normal. Liver and spleen scans showed slight hepatomegaly with irregular activity confirming hepatitis. Cultures: throat swab: no haemolytic streptococci. At the time of admission it was felt that the patient had serum sickness, and acute rheumatism was considered. Treatment with high doses of penicillin was started. However, after 5 days the liver function was disturbed (SGOT 200 U/l; SGPT 652 U/l; alkaline phosphatase 72 U/l; γGT 62 U/l) and moreover HBsAg was present in the serum while no HBsAb could be detected. So it was evident that our patient was suffering from acute viral hepatitis B. At this time the urticaria was not more evident. Complement consumption was noted: C3 50% and C4 9%. The treatment with penicillin was withdrawn and bed rest was advised. Subsequently liver function became normal and the patient recovered well.

Case 2. A 21-year-old man was admitted to our hospital, because of a 4 days history of redness and pruritus, followed by fever, general malaise, arthritis and generalized urticaria. He had taken narcotics intravenously before the onset of his illness.

Physical examination revealed generalized urticaria, tenderness of his left ankle and the liver edge was tender and palpable 2·5 cm below the right costal margin. There was no lymphadenopathy.

Laboratory studies disclosed the following values: WBC $6{\cdot}2 \times 10^9$/l with normal differential, Hb 10·1 mmol/l; ESR 11 mm/h; urinalysis normal except for the presence of urobilin and bilirubin. Serum creatinine 74 μmol/l; SGOT 117 U/l; serum alkaline phosphatase 138 U/l and γGT 60 U/l. In his serum HBsAg was positive but no HBsAb was present.

Controls

Seven patients with idiopathic urticaria and negative for HBsAg and HBsAb in blood served as a control group.

Immunofluorescence studies

Punch biopsies were taken from the lesional and clinically uninvolved skin under local anaesthesia (1% lidocaine). The specimens were prepared following the technique described earlier (Cormane, Szabo & Hauge, 1970), which involves quick freezing in liquid nitrogen and storing at −70°C. Sections were cut at 4 μm thick with a cryostat (International Equipment Co., Washington DC, USA) and dried on a microscope slide for 30 min. Next they were washed with PBS (pH 7·2) for 30 min and then incubated with conjugates in a moist chamber for 30 min. Finally they were washed twice for 30 min with PBS (pH 7·2).

Conjugates. Commercially prepared FTC-labelled antisera were used:
Goat antihuman IgG, lot no. 2257U001A*.
Rabbit antihuman IgA, batch no. KH 14-15-F01†.
Rabbit antihuman IgM, batch no. KH 15-18-F01†.
Rabbit antihuman C1q, batch no. KH 43-02-P06† (this antiserum is not labelled second step with: goat antirabbit Ig, batch no. GAR: FITC 34-1078‡).
Horse antihuman C3 (C3b and C3c), batch no. F 124, H011-5§.
Rabbit antihuman HBsAg, lot no. 924 A¶.
Working dilutions were 1/60 and 1/120.

Fluorescence microscopy

A Leitz Orthoplan microscope with a Xenon XBO-75 lamp and a Leitz vertical illuminator was used. FTC fluorescence was detected using the filter combination BG 38 (4 mm), KP 490 (2 mm) and a secondary filter OG 515 (3 mm). Leitz water immersion objectives (×50 and ×90) were used in all cases.

Histopathology

Tissue for light microscopic examination was fixed in 10% formaldehyde solution and stained with haematoxylin and eosin after paraffin embedding.

RESULTS

IF studies

In the involved skin (urticarial lesions) of both patients with viral hepatitis B, HBsAg, C1q and C3 deposits were detectable in a granular pattern in the small superficial blood vessels (Fig. 1). No immunoglobulin deposits could be demonstrated. No deposits were seen in the uninvolved skin of the patients. All seven controls were negative.

Histopathology

In the dermis a poor perivascular infiltrate of mainly lymphoid cells and a few eosinophilic granulocytes was present. There was slight oedema of the papillary dermis.

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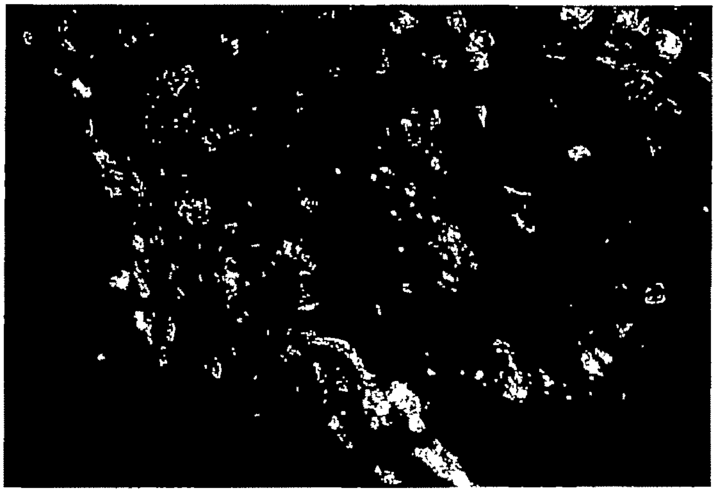

FIGURE 1. Deposits of HBsAg in the blood vessel wall of the dermis

DISCUSSION

Urticaria associated with acute viral hepatitis is, especially in the prodromal phase, a well known phenomenon (Ljungren & Möller, 1971; Lockshin & Hurley, 1972; Monroe & Jones, 1977). Other skin manifestations such as purpura, various erythemas (Veyre & Brette 1975) and cutaneous necrotizing venulitis (Dienstag *et al.*, 1978) have been reported.

In the prodromal phase of viral hepatitis a serum sickness-like syndrome, characterized by general malaise, fever, skin rashes (e.g. urticaria), polyarthralgia and sometimes arthritis is often seen. Circulating immune complexes could be demonstrated in this serum sickness syndrome associated with hepatitis B infection (Lieberman, Rice & Malette 1977; Wands *et al.*, 1975). HBsAg containing immune complexes were found in the glomerulus (Combes, 1971), in skeletal muscle from a patient with polyarteritis (Goche *et al.*, 1970) and in the synovia of inflamed joints (Onion, Crumpachers & Gilliland, 1971). Our results provide evidence that the urticarial lesions in the early phase of viral hepatitis B infection are also induced by HBsAg containing immune complexes. Because of the presence of C1q in the blood vessel walls it seems that complement activation takes place by the classical pathway, which is suggestive of the presence of immune complexes. A number of RNA viruses, Moleney leukaemia virus and vesicular stomatitis virus consume complement factors without antibodies (Bartholomew & Esser, 1980; Beebe, Mills & Cooper, 1980; Cooper *et al.*, 1976; Welsh *et al.*, 1975). There are, as far as we know, no data showing that hepatitis B virus can bind C1q directly. Although no immunoglobulins could be detected, the demonstration of HBsAg, C1q and C3 in the small vessel walls suggests the presence of HBsAg HBsAb complexes. It is of note that HBsAg could be detected only in the lesional skin.

Our results confirm the observations of Dienstag *et al.* (1978) and support the hypothesis that the skin manifestations of viral hepatitis are caused by HBsAg containing complexes. Lockshin & Hurley (1972) suggested that the urticarial lesions are induced by antigens from the inflamed liver. These antigens would form complexes and cause urticarial eruptions by mast cell activation. However, the demonstration of HBsAg in the skin lesions makes us believe that the virus particle itself plays a major role. In contrast to Dienstag *et al.* (1978) our light microscopic study shows no necrotizing vasculitis.

The negative immunofluorescence results in the control patients confirm the results of Synkowski *et al.* (1979), who could not demonstrate immune complexes in a group of urticaria patients. After infection with hepatitis B virus, at least three different antigens may appear in the serum (surface-'s'-, core-'c'- and 'e' antigen). The patient will form antibodies in the following sequence: anti HBc, anti HBe and anti HBs (Aikawa *et al.*, 1978). As a consequence different antigen-antibody complexes will be formed which may induce a variety of hepatic and extra-hepatic disorders. Further studies are necessary to detect a possible relationship between the different antigens and special clinical manifestations.

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Distribution and degradation of albumin in extensive skin disease

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SUMMARY

The distribution and degradation of albumin were determined in twelve patients with extensive skin disease and in ten control subjects by measuring the metabolic turnover and transcapillary escape of ^{131}I-labelled albumin. The ratio of intravascular to total mass of albumin was normal. Thus the observed hypoalbuminaemia and the low intravascular mass reflect a reduced mass of total body albumin. The rate of synthesis was normal, but the transcapillary escape rate reflecting the microvascular leakiness to macromolecules, and the fractional disappearance rate were significantly higher in the patients than in the controls ($P<0{\cdot}001$). It is concluded that the hypoalbuminaemia in these patients is the result of an increased endogenous catabolism of albumin without significant loss via urine, stools or skin. A positive correlation between the transcapillary escape rate and fractional catabolic rate of albumin supports the concept of a causal relationship between these parameters.

Hypoalbuminaemia is a common finding in patients with extensive skin disease (Abrahams, McCarthy & Sanders, 1963; Shuster & Wilkinson, 1963; Parving, Worm & Rossing, 1976; Worm & Rossing, 1980). In recent studies it was accompanied by a low or normal plasma volume thus reflecting a reduced intravascular mass of albumin (IVM) (Parving *et al.*, 1976; Worm & Rossing, 1980). The cause might be displacement of albumin from intravascular to extravascular compartments or a reduction of total body mass of albumin. The displacement theory might find support in the fact that the endothelium of the microvasculature is abnormally leaky to macromolecules in these patients (Marks & Shuster, 1966; Shuster & Marks, 1970; Marks, Birkett & Shuster, 1972; Parving *et al.*, 1976; Worm & Rossing, 1980). When this leakiness was normalized after treatment with systemic steroid, IVM remained low (Parving *et al.*, 1976) indicating that other mechanisms such as loss or increased catabolism are involved.

The aim of the present study was to further elucidate this by albumin metabolic studies.

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MATERIAL AND METHODS

Six male and six female patients with extensive skin disease were examined after having given informed consent. Patient data and diagnoses are listed in Table 1. The patients had no concomitant diseases as judged by routine laboratory blood values, urine, chest X-ray, blood pressure, ECG or clinical examinations. Only bland topical treatment was applied during the examination period. Five of the patients had erythroderma. It was idiopathic in three patients (nos. 1–3) and due to psoriasis in two (nos 4 and 5). None of the patients had bullous diseases.

The metabolism of albumin was determined as in a previous report (Rossing, 1967) using ^{131}I-albumin (Kjeller, Norway) as the tracer. This preparation is of a satisfactory metabolic quality (Rossing & Jensen, 1967).

The patients were studied in the morning after at least 12 h of fasting and 30 min of rest in the supine position. About 20 μCi of the albumin tracer was injected intravenously. Venous blood samples were drawn from the other arm 10 min later and seven more times during the first 60 min after injection by syringes without stasis. Blood sampling was continued daily for the next week and every second day for the following week. Whole-body counting was carried out, on the same days as the blood sampling, with a gammacamera (Radicamera 60, General Electric) without collimator, equipped with a whole-body scanning system. The thyroid gland was blocked throughout the period of study by potassium iodide 100 mg daily to secure renal clearance of all ^{131}I liberated from the tracer albumin on its degradation.

The activity for all plasma samples was divided by the plasma albumin concentrations (Mancini, Carbonara & Heremans, 1965) to avoid errors due to accidental changes in plasma volume distribution.

Calculations

The transcapillary escape rate of albumin (*TER*), i.e. the fraction of intravascular albumin passing to the extravascular space per unit time, is calculated from the initial part (60 min) of the plasma

TABLE 1. Data and diagnosis of twelve patients with extensive skin disease

Case no.	Sex	Age (years)	Height (cm)	Weight (kg)	Surface area (m²)	Diagnosis	Skin involvement (%)
1	M	64	170	80	1·90	Erythroderma	Generalized
2	F	77	160	118	2·04	Erythroderma	Generalized
3	F	58	167	72	1·80	Erythroderma	Generalized
4	M	58	173	78	1·92	Erythroderma	Generalized
5	M	53	182	100	2·19	Erythroderma	Generalized
6	M	71	164	61	1·67	Contact dermatitis	Generalized
7	M	69	174	85	1·98	Contact dermatitis	80
8	F	34	155	55	1·53	Pustular psoriasis	40
9	F	64	165	95	1·95	Pustular psoriasis	50
10	M	80	169	80	1·89	Psoriasis	90
11	F	43	171	74	1·87	Psoriasis	80
12	F	62	162	90	1·88	Psoriasis	75
Patients	Mean	61	168	82	1·89		
	±s.d.	13	7	17	0·17		
Controls	Mean	45	172	68	1·81		
(n = 10)	±s.d.	16	7	12	0·17		

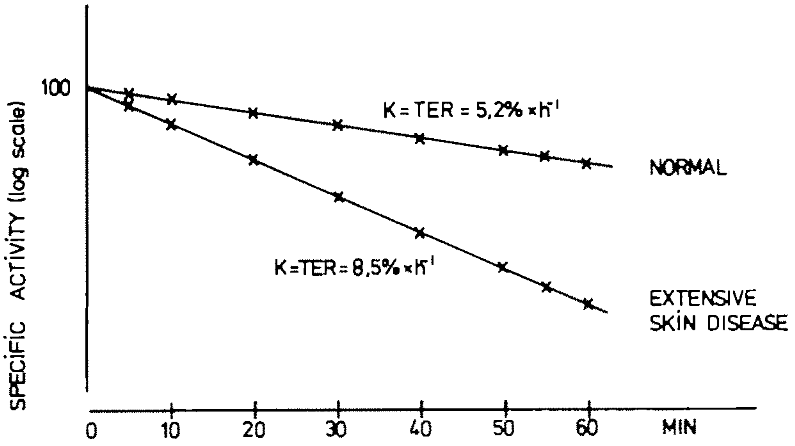

FIGURE 1. Typical decrease in plasma specific activity of ^{131}I-albumin during the first 60 min after injection in a control subject and in a patient with extensive skin disease. TER = trans-capillary escape rate.

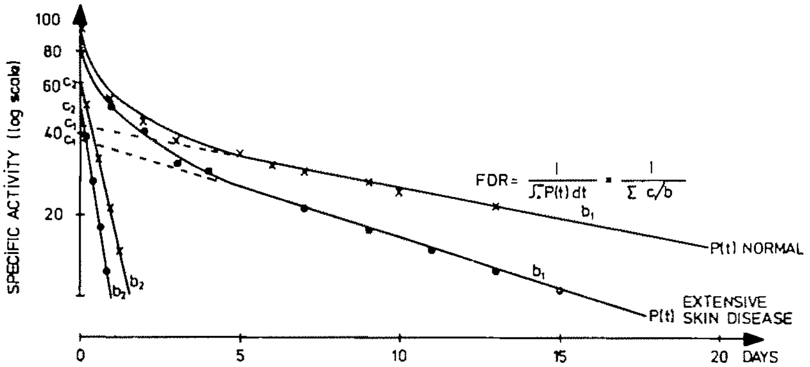

FIGURE 2. Typical plasma activity curves in a control subject and in a patient with extensive skin disease. FDR equals the fractional disappearance rate, P(t) the plasma curve, t the time, c the intercepts and b the rate constants.

disappearance curve after injection of the albumin tracer as calculated by the least squares' method from the first eight plasma samples (Fig. 1). The investigative procedure was exactly the same as in previous reports (Worm & Rossing, 1980). The theoretical basis for the calculation has been described in detail previously (Parving & Gyntelberg, 1973).

PV was calculated by extrapolating the initial part of the plasma disappearance curve and from the injected dose of tracer albumin.

IVM equals the product of PV and plasma albumin concentration.

The remaining metabolic variables were calculated according to Nosslin (1965) by graphic resolution of the total tri- or bi-exponential plasma disappearance curve drawn on semilog scale. The final slope of the plasma curve was drawn parallel to the monoexponential whole-body curve (Rossing, 1969).

The necessary assumptions for obtaining correct results by this technique are, that (1) the catabolism of endogenous albumin occurs within or close to the vascular bed, and (2) the albumin metabolism is in a steady state. The first condition is discussed elsewhere (Rossing, 1967; Rothschild, Oratz & Schriber, 1972). The patients showed no systematic changes in haematocrit, plasma protein concentrations or body weight during the study. This was taken to indicate metabolic steady state.

The fractional disappearance rate (*FDR*) of albumin is the fraction of IVM disappearing by loss to the exterior and endogenous catabolism per 24 h (Fig. 2) determined by graphic 'peeling off' of the total plasma curve: $1/\int_0^\infty P(t)dt = 1/\Sigma\ c/b$, where P(t) is the normalized intravascular activity, t the time, each c value an intercept and each b value a rate constant.

The metabolic turnover rate of albumin (*S*) equals the rate of synthesis, if the assumption of steady state is fulfilled, whereafter $S = \text{IVM} \times \text{FDR}$.

The distribution ratio (*D*) is the per cent of the total albumin mass located intravascularly. D is calculated from the mean sojourn time in plasma and the mean sojourn time in the total body $= (\int_0^\infty P(t)dt)^2/\int_0^\infty t \cdot P(t)dt = (\Sigma\ c/b)^2/\Sigma\ c/b^2$.

Statistics

The results were compared with those obtained in ten normal control subjects (six male and four female) by the same technique. Student's t-test was used for statistical evaluation.

RESULTS

The mean plasma albumin concentration, IVM and TM of the patients were significantly reduced compared to the mean values of the control group ($0{\cdot}05 > P < 0{\cdot}01$) (Table 2).

The PV of the patients was significantly reduced when related to weight (36·4 ml/kg ± 5·8 s.d.)

TABLE 2. Plasma albumin concentration (Alb), plasma volume (PV), intravascular and total mass of albumin (IVM and TM), transcapillary escape rate of albumin (TER), fractional disappearance rate (FDR), turnover rate (S), and distribution ratio (D) in twelve patients with extensive skin disease

Case no.	Alb (g/l)	PV (ml)	IVM (g)	TM (g)	TER ($\%$ IVM $\times h^{-1}$)	FDR ($\%$ IVM $\times 24\ h^{-1}$)	S ($g \times 24\ h^{-1}$)	D ($\%$)
1	37·8	2829	107	289	9·5	20·7	22·1	37
2	28·3	2956	84	200	8·6	19·5	16·4	42
3	35·5	2565	91	202	8·6	17·2	15·7	45
4	22·8	3211	73	166	8·0	16·9	12·4	44
5	29·3	4065	119	271	8·0	13·7	16·3	44
6	37·2	2516	94	240	7·5	17·0	15·9	39
7	31·0	3391	105	233	6·7	15·2	16·0	45
8	22·4	2076	47	126	9·2	17·5	8·1	37
9	32·1	2920	94	224	7·5	15·2	14·2	42
10	30·6	3499	107	243	8·3	14·0	15·0	44
11	28·2	2769	78	135	7·3	14·0	10·9	58
12	36·8	2500	92	224	7·5	17·3	15·9	41
Patients Mean	31·0	2941	91	213	8·1	16·5	14·9	43
±s.d.	5·2	536	19	50	0·8	2·2	3·4	5
Controls Mean	40·0	2951	118	285	5·2	11·7	13·7	42
(n = 10) ±s.d.	2·6	682	27	67	0·7	1·1	2·5	2
	$P<0{\cdot}05$	N.S.	$P<0{\cdot}05$	$P<0{\cdot}01$	$P<0{\cdot}001$	$P<0{\cdot}001$	N.S.	N.S.

N.S. = not significant.

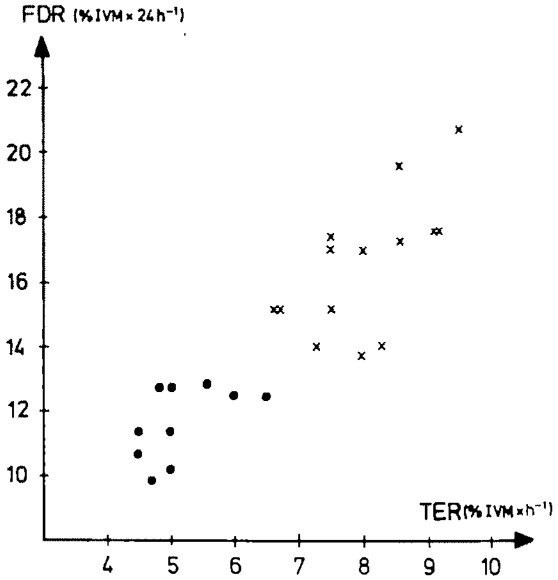

FIGURE 3. Correlation between transcapillary escape rate (TER) and fractional disappearance rate (FDR) of albumin (r = 0·86, $P<0·001$). (×) Patients with extensive skin disease; (o) control subjects.

($P<0·01$), but not when related to height (17·4 ml/cm±2·5 s.d.) and surface area (1·55 l/m^2±0·18 s.d.) or expressed as the total volume (2941 ml±536 s.d.) compared to the control values (43·2 ml/kg± 4·3 s.d., 17·2 ml/cm±3·4 s.d., 1·62 l/m^2±0·23 s.d. and 2951 ml±682 s.d. respectively).

TER and FDR were significantly increased in the patients compared to the controls ($P<0·001$) (Table 2). There was a positive linear correlation between TER and FDR in the patients (r = 0·62, $P<0·05$). This correlation is more pronounced when the TER and FDR values from the controls are included ($r=0·86$, $P<0·001$) (Fig. 3).

Metabolic turnover rate (S) and intravascular to total albumin ratio (D) were normal (Table 2).

DISCUSSION

The low plasma albumin concentration and the even more pronounced decrease in IVM in patients with extensive skin disease have been reported previously (Parving *et al.*, 1976; Worm & Rossing, 1980). The mechanisms responsible for these findings become more evident from the present metabolic turnover data. It can now be concluded that low IVM is caused neither by dilution nor by extravascular displacement as suggested by others (Shuster & Marks, 1970) but reflects a low total mass of albumin in the body. The extravascular mass of albumin (EVM) is decreased to the same extent as the IVM.

There are some discrepancies between these results and those obtained by Shuster & Wilkinson (1963) in similar patients and later discussed by Shuster & Marks (1970). They suggested that the most important factors in the development of hypoalbuminaemia are intravascular dilution and expansion of the extravascular albumin space, whereas the size of the total albumin pool was judged to be normal. This discrepancy may be due to different methods applied. Their way of calculating the distribution ratio (Sterling, 1951) leads to an underestimation resulting in EVM values inappropriately high.

The main conclusion from the study of Shuster & Wilkinson (1963) namely that patients with extensive skin disease have a shortened half-life of intravascular albumin agrees, however, completely with our finding of an increased fractional disappearance rate (FDR) (i.e. shortened sojourn time in plasma). Recently also Braverman (1979) reported that patients with severe pustular psoriasis and hypoalbuminaema have a shortened half-life of intravascular albumin thus confirming an earlier report (Braverman, Cohen & O'Keefe, 1972).

The disappearance rate of albumin is determined by the catabolic rate and—hypothetically—by a pathological loss to the exterior. In contrast to some reports (Shuster & Wilkinson, 1963; Cotterill, Cunliffe & Bulusu, 1971) but in agreement with others (Braverman, 1979; Braverman *et al.*, 1972; Pegum, 1951) we have demonstrated neither proteinuria nor protein-losing gastroenteropathy in patients with extensive skin disease in recent studies (Parving *et al.*, 1977; Worm, 1981).

It is well known, that patients with burns have an extensive loss of plasma albumin through the burned skin, the loss being correlated to the extent of the burn (Birke *et al.*, 1968). There must be a rather considerable oozing of interstitial fluid in burned patients in the absence of an epidermal barrier, the coexisting increased capillary permeability furthermore supporting the loss of plasma proteins (Birke *et al al.*, 1968). Likewise it has been suggested that loss of albumin through the skin is partly responsible for the hypoalbuminaemia in patients with extensive skin disease due to desquamation, especially in eczema and psoriasis and oozing through an inflamed skin surface. Most of the protein lost by desquamation is keratin (Baden & Freedberg, 1979), and the experimental evidence for the loss of plasma proteins via the skin is scarce (Shuster & Wilkinson, 1963; Pegum, 1951). Freedberg & Baden (1962) found that even gross exfoliation was followed by neither negative nitrogen balance nor clinical manifestations of protein depletion.

In the present study we kept record of the urinary excretion of non-protein bound ^{131}I in one patient with a marked increase in plasma disappearance rate (case 1). In this patient endogenous catabolism and not loss to the exterior was responsible for the disappearance of all tracer albumin. This technique depends totally on complete urine collection, and that may explain the discrepancy between plasma and urinary activity in some patients in the study of Shuster & Wilkinson (1963), although they found activity in the scales in one patient.

It must therefore be justified to state that loss of plasma albumin via the skin is not a regular feature in patients with extensive skin disease.

The plasma albumin concentration is the resultant of several factors such as rate of catabolism, rate of synthesis and distribution (Rothschild *et al.*, 1972). With a normal distribution and rate of synthesis, the latter even being slightly increased, we must conclude that the true cause of the hypoalbuminaemia is the increased endogenous catabolism, and that the fractional disappearance rate equals fractional catabolic rate (FCR).

A significant positive correlation between TER and FDR (i.e. FCR) as demonstrated in this study (Fig. 3) agrees completely with some of the studies in patients with increased microvascular leakiness to albumin, due to different disease entities (Parving, Rossing & Sander, 1975). This correlation may reflect a causal relationship between the passage of albumin through the microvascular wall and the catabolism. This hypothesis was first put forward by McFarlane (1963) and later by Rossing, Krasilnikoff & Christiansen, (1972). The functional and structural changes in the microvasculature responsible for the increased leakiness may, however, vary from one disease to another. In a recent paper (Worm & Rossing, 1980) we have suggested that the open junctions of the dermal venular endothelium, demonstrable in psoriasis (Braverman, 1972) and in inflammatory reactions (Majno, Palade & Schoefl, 1961), are responsible for the increased leakiness to macromolecules in extensive skin disease.

The transendothelial albumin flux is essentially unidirectional, the interstitial macromolecules being drained by the lymph (Noer & Lassen, 1978). A normal distribution of albumin in spite of an increased extravasation must therefore imply a compensatory increase in lymphatic return rate (LRR) of albumin as TER × IVM = LRR × EVM in steady state conditions. This mechanism has also been described in other diseases with increased microvascular leakiness of albumin (Parving *et al.*, 1975) and fits in with earlier direct measurements of the subcutaneous albumin absorption (Langgård, 1963; Hollander, Reilly & Burrows, 1961).

Direct estimation of the lymphatic function in skin diseases has been the subject of only a single study (Ellis, Marks & Perry, 1970) in which the disappearance rate of radiolabelled albumin from the dermis was about the same in different skin diseases and in the normal skin. The main finding was, however, a striking regional variation in the clearance rate.

In conclusion the hypoalbuminaemia in patients with extensive skin disease is the result of a shortened intravascular sojourn time of albumin, which at least in some marked cases is due solely to an increase endogenous catabolism, although loss via the diseased skin cannot always be excluded.

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Photosensitization of *Trichophyton* with 8-methoxypsoralen to UV-A light radiation

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SUMMARY

The photosensitization effect of 8-methoxypsoralen (8-MOP) and subsequent long-wavelength ultraviolet (UV-A) irradiation on *Trichophyton mentagrophytes* was examined by *in vitro*, semi-*in vivo*, and *in vivo* experiments. A marked fungicidal effect of 8-MOP plus UV-A (PUVA) on suspensions of fungal spores was observed *in vitro* as measured by loss in colony-forming ability of *T. mentagrophytes*. 8-MOP application or UV-A irradiation alone did not show such an ability. The antifungal effect of PUVA was also observed on the infected scales obtained from experimental and clinical dermatophytosis. The *in vivo* activity of PUVA treatments was also demonstrated on the lesions of experimentally induced dermatophytosis in guinea-pigs. These observations suggest possible clinical applications of photochemotherapy on superficial fungal infections.

The furocoumarins (psoralens) are well known to photosensitize various biological systems to long wavelength ultraviolet light (UV-A) irradiation both *in vitro* and *in vivo*. Photoactive psoralens form covalent mono- and bi-additions (cross-linkings) with pyrimidine bases in double-stranded DNA in the presence of UV-A (Musajo, Rodighiero & Dall'Acqua, 1965; Musajo *et al.*, 1967; Musajo, Bordin & Bevilaoqua, 1967). The structural changes in the DNA strand interfere with normal DNA replication and account for the inhibition of scheduled DNA synthesis (Baden *et al.*, 1972). This may be followed by suppression of cell division. The mechanism by which psoralen plus subsequent UV-A exposure (PUVA) exerts therapeutic benefit in various dermatoses, especially in psoriasis, seems to be explained by these experimental observations. Lethal or mutagenic photosensitizing effects of PUVA have been demonstrated in bacteria, viruses, *Candida albicans* and cultured mammalian cells (Oginsky *et al.*, 1959; Cole, 1970; Daniels, 1965; Ben-Hur & Elkind, 1973). The purpose of this report is to present additional data on the photosensitization effect of PUVA on *T. mentagrophytes* using various experimental procedures; *in vitro*, semi-*in vivo*, and *in vivo*.

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MATERIALS AND METHODS

Preparation of the fungal suspensions

A zoophilic granular strain of *Trichophyton mentagrophytes* was grown on Sabouraud's dextrose agar slant medium for 2 weeks at 28°C. Suspensions were prepared by flooding the surface of the medium with sterile water and gently removing the colony with a wire loop. Clumps of removed fungus were ground with a mortar and pestle for 10 min and the suspensions were agitated vigorously for 10 min in a vortex mixer and filtered through eight layers of gauze. The filtrates were washed with sterile water three times by centrifugation at 3000 rpm for 10 min. The spores in the preparation were counted using a haemocytometer and diluted to give the desired densities.

Light sources

The light source, a fluorescent black light (Toshiba FL 20S BLB, Toshiba Medical Supply, Tokyo) emitting rays between 300 and 420 nm (mainly UV-A light peaking at 360 nm) was used, and five lamps were housed in a reflector unit. The irradiance of UV-A (320–400 nm) and UV-B (shorter than 320 nm) which was measured by a photovoltaic cell (UVR-305/365 Radiometer, Toshiba Medical Supply, Tokyo) through a plastic Petri dish cover was 3·0 mW/cm^2 and 0·45 mW/cm^2, respectively, at a distance of 10 cm.

In vitro experiments

0·25 ml of 1% 8-methoxypsoralen (8-MOP) in ethanol: acetone: propylene glycol (7: 2: 1 vols) was added to 1 ml of each fungus suspension, which contained 4×10^5, 4×10^4, or 4×10^3 spores, giving a final concentration of 0·2% 8-MOP. In a preliminary examination, 8-MOP in higher concentrations revealed some inhibitory effect on fungal growth even in the absence of UV-A irradiation. Therefore, this admixture ratio was chosen. Ten min after the admixing, 0·01 ml of each mixture (containing 3200, 320, or 32 spores in 0·2% 8-MOP) was dropped onto Sabouraud's dextrose agar medium in a Petri dish. Ten min after application, fungus on the medium was irradiated with black light through the plastic cover of the Petri dish. Three dosage levels of UV-A (2·7 J/cm^2, 5·4 J/cm^2, and 10·8 J/cm^2) were used for the present study. After irradiation Petri dishes were wrapped with aluminium foil and cultured at 28°C. Identical plates without either 8-MOP or irradiation, with only irradiation, with only 8-MOP, and with vehicle and irradiation served as controls. The number of inoculated spores on control plates was the same as on PUVA-plates. These experiments were carried out in triplicate.

Semi-in vivo experiments

Guinea-pigs. Experimental dermatophytosis was induced in guinea-pigs according to the inoculation method of Tagami, Watanabe and Ofuji (1973). Hartley strain albino guinea-pigs, weighing 400–500 g, were used. The skin of the mid-back of three guinea-pigs was prepared by depilating and subsequently clipping any remaining hairs. 0·01 ml of the spore suspension, containing 40,000 spores, was delivered to the inoculation site with a micropipette. The site was immediately covered with a 2 × 2 cm sheet of polyethylene film and the inoculum was spread by light pressure on the film, which was secured with an impermeable plastic tape (Blenderm, Mining & Manufacturing Co., Minnesota) and held in place by elastic adhesive bandage 3·8 cm in width (Elatex, Eizai Co., Tokyo). The occlusive dressing was left in place for 24 h. All the inoculated guinea-pigs developed an infiltrated erythematous plaque covered by thick silvery scales within 1 week.

The semi-*in vivo* antifungal effect of 8-MOP plus UV-A was evaluated by a modification of the procedure of Dolan *et al.* (1957). Two weeks after inoculation, the scales were removed with a blunt scalpel from the lesions of three infected guinea-pigs. Fungal hyphae were confirmed by microscopy in all KOH mounts of the scales examined at random. Part of the scale scrapings was immersed in commercially available 1% 8-MOP solution (Oxsoralen Lotion, Taisho Pharm. Co., Tokyo) for 10 min. The scales were removed and placed on blotting paper. One hour later the scales were irradiated with 18 J/cm^2 of UV-A by black light, and then were cultured on Sabouraud's dextrose agar medium. Scales exposed to 8-MOP only, to UV-A only, and to neither treatment served as controls. These cultures were performed in triplicate.

Human. Roofs of blisters ranging from 5 to 10 mm in diameter were excised by scissors from 5 patients with tinea pedis. These samples were divided into four pieces with a razor blade. Each quarter of the material was cultured after one of the following treatments; (1) PUVA, (2) 8-MOP immersion, (3) UV-A irradiation, or (4) no treatment. The procedures of 8-MOP immersion and UV-A irradiation were identical to those used for the scales from experimental animals.

In vivo experiments

Experimental dermatophytosis was induced in ten guinea-pigs by procedures identical to those used in the semi-*in vivo* experiments except that two anatomically symmetrical sites on the back were infected. On the third postinfective day, PUVA treatment was started on the right sided inoculation sites. It was performed three times a week by topical application of 1% 8-MOP solution 2 h prior to UV-A irradiation at a dose of 5·4 J/cm^2. The other inoculated side received the same amount of UV-A without 8-MOP application.

RESULTS

In vitro experiments

The results of culture for 1 week are summarized in Table 1. The control fungus suspensions in distilled water revealed excellent growth at each density of spores. On the other hand, UV-A irradiation in the presence of 8-MOP resulted in a marked photosensitization effect on the fungus (Fig. 1). No fungal growth was observed on the medium plates on which thirty-two and 320 spores were inoculated after the PUVA treatments. The UV-A dose of 2·7 J/cm^2 was sufficient to have a lethal

TABLE 1. Results of culture of *T. mentagrophytes* after various treatments

	Numbers of spores cultured		
Treatment	3200	320	32
H_2O	+++	+++	+++*
8-MOP	+++	+++	+++
H_2O-UVA-2·7 J/cm^2	+++	+++	+++
H_2O-UVA-5·4 J/cm^2	+++	+++	+++
H_2O-UVA-10·8 J/cm^2	+++	+++	++
Vehicle-UVA-10·8 J/cm^2	+++	+++	++
PUVA-2·7 J/cm^2	+	−	−
PUVA-5·4 J/cm^2	−, +	−	−
PUVA-10·8 J/cm^2	−, +	−	−

* Diameter of colony after 1 week culture.
+++ >2 cm; 2 cm> ++ > 1 cm; 1 cm> + >0; − = no growth.

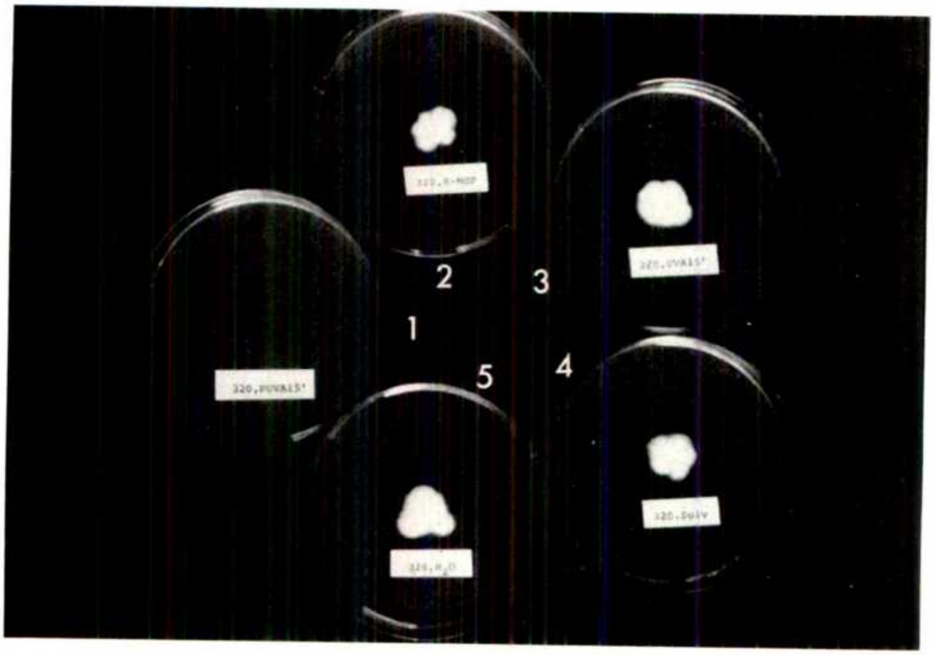

FIGURE 1. One-week culture of 320 spores of *T. mentagrophytes* treated with (1) PUVA, (2) 8-MOP; (3) 2·7 J/cm² of UVA, (4) vehicle plus UVA, and (5) water.

TABLE 2. Culture of blister roofs from tinea pedis

Patient	No treatment	UVA	8-MOP	PUVA
1	+ + +	+ + +	+ + +	—*
2	+ + +	+ +	+ + +	—
3	+ + +	+ + +	+ + +	+
4	+ + +	+ + +	+ + +	+
5	+ + +	+ + +	+ + +	+

* Diameter of colony after 1 week culture.
+ + + > 1 cm; 1 cm > + + > 0·5 cm > + > 0 cm; — = no growth.

effect on these densities of spores. In contrast, UV-A irradiation as much as 10·8 J/cm² was insufficient to kill 3200 spores completely even in the presence of 8-MOP, although an inhibitory effect on fungal growth was quite evident. All three plates to which 3200 spores were inoculated and 2·7 J/cm² of PUVA was given showed much less fungal growth than control plates. When the UV-A dose was increased, a complete killing effect of PUVA was observed on one and two of three plates exposed to 5·4 J/cm² and 10·8 J/cm², respectively. Almost no detectable effect on fungal growth was observed with 8-MOP in the absence of UV-A, with UV-A in the absence of the compound, or with UV-A in the presence of vehicle. Only a mild inhibitory effect of UV-A was noted on the plates on which thirty-two spores were inoculated. The plates which had not shown fungus colony in 1 week did not reveal it thereafter.

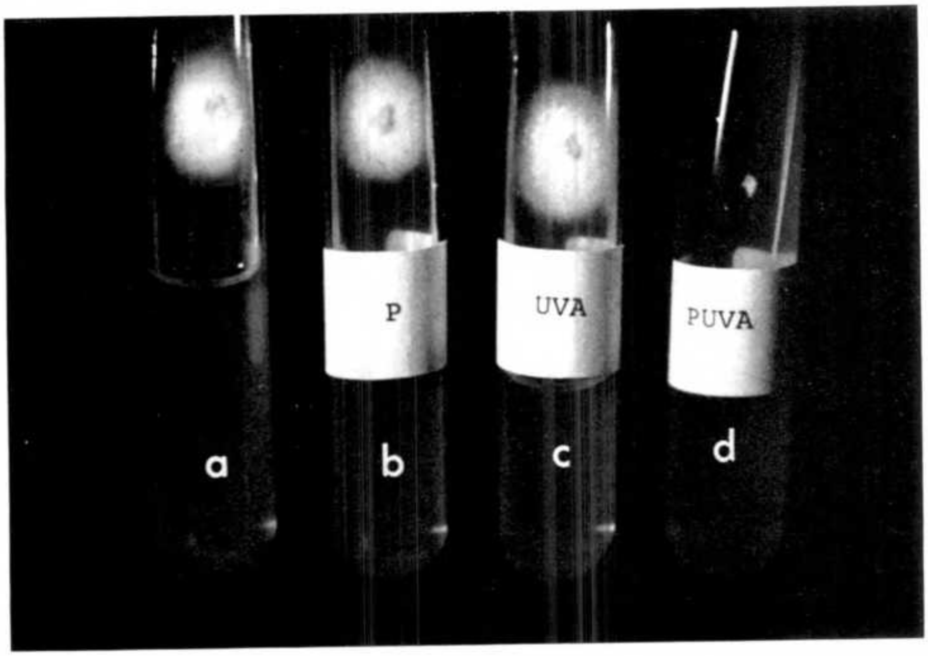

FIGURE 2. One-week culture of blister roof from tinea pedis; (a) no treatment, (b) 8-MOP immersion, (c) UVA irradiation, (d) PUVA.

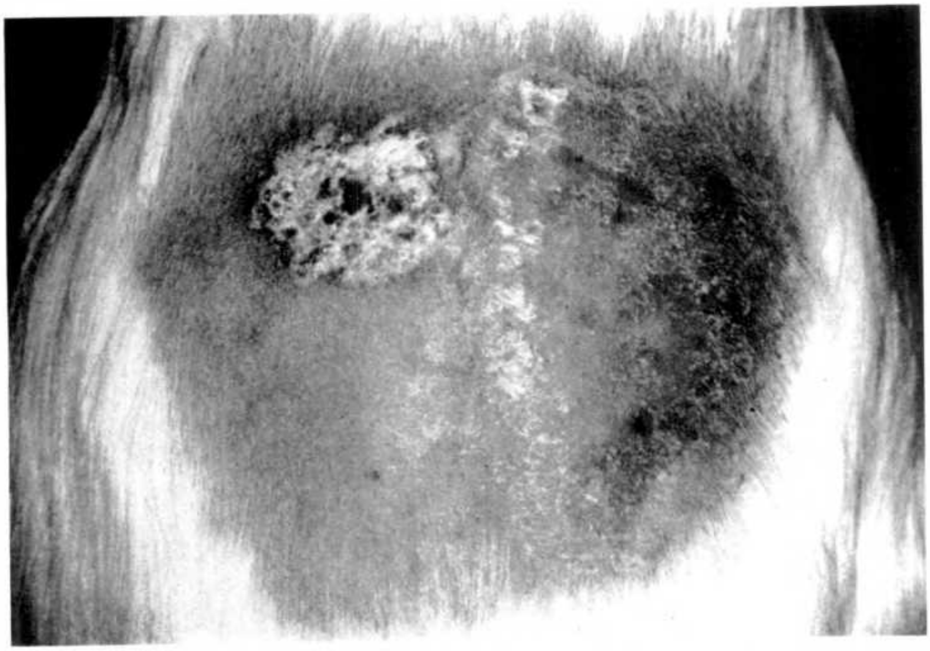

FIGURE 3. Lesions of experimentally induced dermatophytosis on 15th postinfective day. Left side was irradiated with only UVA and right side was treated six times with PUVA.

Semi-in vivo experiments

The non-treated scales obtained from the experimental dermatophytosis in guinea-pigs revealed excellent fungal growth. On the other hand, the scales which had been irradiated with UV-A in the presence of 8-MOP showed much less growth than controls, while a complete killing effect was not observed. Neither 8-MOP in the absence of UV-A irradiation nor UV-A irradiation in the absence of 8-MOP showed inhibitory effects on fungal growth.

Similar results were obtained with the materials taken from five patients with tinea pedis (Table 2). A complete killing effect of PUVA was attained in two samples (Fig. 2), while only an inhibitory effect was observed in the remaining three samples. *T. mentagrophytes* was cultured from blisters of all patients.

In vivo experiments

All guinea-pigs which had been inoculated with *T. mentagrophytes* developed papulo-erythematous lesions confined to the inoculation sites on the third postinfective day, when PUVA treatment was initiated. The lesions became more marked in a few days, with the formation of scaly, infiltrated, erythematous plaques. There was no difference in the severity of the lesions between UV-A and PUVA treated sites after two treatments, that is, until the 5th postinfective day. The most intense reaction was observed between the 7th and 14th postinfective day at the control inoculation sites which had been irradiated with UV-A in the absence of 8-MOP. Most of the animals showed milder lesions in PUVA-treated sites in these periods (Fig. 3). The improvement in the PUVA-treated lesions was apparent after five treatments, with thinning of plaques and decreased scaling. The opposite control site developed infiltrated erythematous plaque covered by thick silvery scales with areas of crusting and oozing.

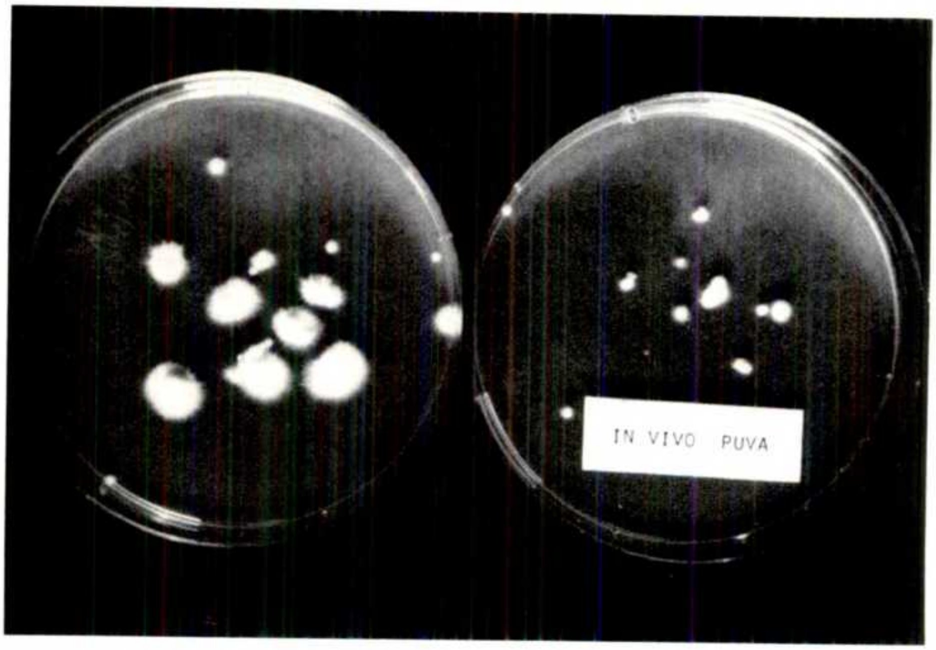

FIGURE 4. Culture of scales from infected guinea-pigs. Materials taken from lesions exposed to only UVA (left) and those from lesions treated eight times with PUVA (right).

Microscopic examination revealed fungal hyphae in KOH mounts of the scales from both sides. However, fungal elements were found in PUVA-treated lesions only with difficulty. Fungus culture also revealed less growth in the scales removed from PUVA-treated lesions than in those from UVA-irradiated control sites (Fig. 4).

The lesions, including control sites, began to resolve by the third week and they regressed completely to leave an alopecic scar by the 4th week. Therefore, the effect of PUVA on the fungal infection could be evaluated only for 3 weeks. Eight treatments were performed during this period. Although complete clearing of lesions by PUVA-treatment was not confirmed, the effect was evident.

DISCUSSION

In the present study, UV-A irradiation to suspensions of *T. mentagrophytes*, in the presence of 8-MOP, resulted in death of the fungus as measured by loss in colony-forming ability. Such irradiation in the absence of 8-MOP or such treatment in the absence of UV-A showed no lethal or inhibitory effect on the fungus. Thus, the effect of 8-MOP on fungal cells is indeed a photosensitization.

In 1961, Mikkelson, Fowlks, and Griffith demonstrated by a paper-disk diffusion method that photosensitization of several common dermatophytic fungi with 8-MOP followed by UV-A irradiation had an effective fungicidal activity *in vitro*. In the present investigation, similar results were obtained *in vitro* using a different procedure. Instead of the paper-disk diffusion method, the organism was suspended in the solution containing the photosensitizing compound. For quantitative examination, hyphal fragments were discarded and only fungal spores were used. We have also observed the fungicidal effect of PUVA on *T. rubrum* and *Microsporum canis* by identical procedures *in vitro* (unpublished observation).

Mikkelson *et al.* (1961) suggested that an investigation of the fungicidal effects of ultraviolet radiation upon dermatophytic fungi after exposure to photosensitizing agents might lead to a treatment of certain chronic fungus infections of human skin. We obtained further experimental evidence suggesting possible clinical applications of photochemotherapy on fungal infections. However, the agents which have fungistatic or fungicidal effects *in vitro* are not always clinically useful because they may fail to reach the infecting organism within the horny layer. Depending upon the environmental conditions, the fungus forms different structures. The predominant form of *T. mentagrophytes* is spore on culture medium and hypha in infected scale. Therefore, therapeutic effects of antifungal agents should be evaluated under conditions which more closely simulate the actual conditions of use than do the *in vitro* methods. For this purpose, Dolan *et al.* (1957) devised a semi-*in vivo* procedure for testing antifungal agents using infected scales obtained from guinea-pigs. By a modification of their method, we also observed antifungal effects of PUVA on scrapings from human as well as from experimental dermatophytosis. This indicates that PUVA is effective not only on spores but also on fungal hyphae. Furthermore, we could demonstrate the *in vivo* activity of PUVA on the lesions of experimentally induced dermatophytosis in guinea-pigs. However, complete clearing by PUVA treatment could not be confirmed in this study, since experimental lesions in animals began to resolve spontaneously by the 3rd week and completely regressed by the 4th week. During this period only eight treatments of PUVA could be carried out. Therefore, clinical study must be performed in humans for longer periods to evaluate the therapeutic efficacy of PUVA. Clinical application of PUVA treatment seems to be possible, since superficial fungus infections of human skin lie within the zone of both ultraviolet and 8-MOP penetrability.

Our current study would indicate another possibility; that photosensitization of *T. mentagrophytes* can be used as a simple laboratory model for assaying phototoxic drug reactions *in vitro* as with *Candida albicans*, viruses, tissue culture cells, or bacteria (Daniels, 1965; Fowlks, 1959; Freeman, Murtishaw

& Knox, 1970; Kobayashi, Wada & Mizuno, 1974). We are presently examining whether phototoxic compounds other than 8-MOP have fungicidal effects on *T. mentagrophytes* in the presence of ultraviolet radiation.

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In vivo effects of PUVA on lymphocyte function

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SUMMARY

Whole-body exposure to erythemogenic doses of PUVA in normal volunteer subjects resulted in a decrease in the proportion of circulating sheep erythrocyte rosette-forming and surface membrane immunoglobulin-positive lymphocytes and an increase in the proportion of null cells. Doses of PUVA resulting in minimal or no erythema had similar but less marked effects on lymphocytes. The changes appeared 30 min after exposure, were maximal at 12–16 h and returned to pre-treatment levels by 72 h post-irradiation. The response of lymphocytes to stimulation with phytohaemagglutinin was unaffected. No alteration occurred in the absolute numbers of circulating lymphocytes or polymorphonuclear leukocytes.

Recent studies have demonstrated that the viability and function of peripheral blood lymphocytes were altered by exposure to UVA (320–400 nm) radiation in the presence of methoxsalen (referred to by the acronym, PUVA). Exposure of these cells to PUVA *in vitro* resulted in a dose-dependent decrease in trypan blue dye exclusion (Morison *et al.*, 1980) and reduction in the incorporation of [^{3}H]-thymidine into nuclear DNA after stimulation with phytohaemagglutinin (PHA) (Scherer, Kern & Braun-Falco, 1977; Kruger, Christophers & Schlaak, 1978; Morison *et al.*, 1980). Exposure to PUVA *in vivo* also altered the function of lymphocytes. S-phase DNA synthesis was depressed immediately following a single *in vivo* exposure to PUVA (Kraemer & Weinstein, 1977). Furthermore, the number and proportion of circulating T lymphocytes, as identified by their ability to form E rosettes, was found to be decreased after repeated exposures to PUVA in patients with psoriasis and in healthy control subjects (Ortonne *et al.*, 1978; Haftek *et al.*, 1979; Cormane, Hamerlinck & Siddiqui, 1979). However, these effects have not been found in all studies; the proportion of E rosette-forming cells in the peripheral blood remained unchanged during a course of PUVA therapy in one study (Morison *et al.*, 1979a) and increased from an initially low level in another study (Fraki, Eskola & Hopsu-Havu, 1979). The influence of the disease state, usually psoriasis, differences in the timing of the *in vitro* studies in relation to the last PUVA treatment, and differences in methodology may account for these variations. The aim of the present study was to examine the acute effects of a single

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exposure to PUVA on the number of peripheral blood polymorphonuclear (PMN) leukocytes and the number and percentage distribution of lymphocyte subpopulations in normal persons. In addition, we tested the effect of a single treatment on certain functions of lymphocytes and sought to determine the time course of any changes observed and assess whether such changes showed a dose-response-relationship to PUVA exposure.

MATERIALS AND METHODS

Informed consent was obtained from fourteen normal subjects. The age range for the group was 22–38 years, with a mean age of 27 years. The study was conducted during the winter months. All subjects were fair-skinned, had a history of a tendency to sunburn and had little or no residual pigmentation from previous sun exposure.

Radiation exposure

The minimal phototoxic dose (MPD) of psoralen/UVA radiation was determined for subjects 1–10 by administering 0·6 mg/kg methoxsalen 2 h prior to irradiating 2 × 2 cm sites with geometrically increasing exposure does of UVA radiation. The lowest exposure dose resulting in delayed erythema at 48 h was designated as the MPD. Three days later, six of these subjects were given a whole-body exposure of 2 × MPD, while the other four subjects were given 1 × MPD. Whole-body irradiation was performed in an upright chamber equipped with sixty-four Sylvania PUVA bulbs. The quantitative spectral characteristics of this radiation source have been published (Levin & Parrish, 1974).

The erythemal response was judged clinically 48 h after exposure on a scale of: 0 = no erythema, ± = faint erythema, 1+ = pink erythema, 2+ = red erythema without oedema, 3+ = fiery red erythema with oedema.

In vitro studies

Twenty to 40 ml of venous blood were collected in a heparinized syringe. After white blood cell and differential counts were performed, the mononuclear cells were separated on a Ficoll–Hypaque gradient and divided for the following tests:

(a) *Enumeration of T cells.* The E rosette test was used as a marker for T lymphocytes. Mononuclear cells were incubated with latex particles (Difco, Detroit, MI) for 45 min to permit identification of monocytes. After washing the cell suspension, an aliquot was mixed with sheep erythrocytes (Becton Dickinson, Cockeysville, MD) plus 10% fetal calf serum (FCS, Gibco, Grand Island, NY) to yield an erythrocyte to lymphocyte ratio of 80:1. The cell suspension was incubated at 37°C for 15 min, centrifuged at 4°C for 5 min at 200 *g* and then incubated at 4°C overnight. Cells were resuspended by gentle tapping of the tube and the rosette-forming lymphocytes (lymphocytes with three or more adherent erythrocytes) counted in a haemocytometer. Cell viability was evaluated by trypan blue dye exclusion; one hundred viable cells were counted, and the number of E rosettes was expressed as a percentage of viable latex-free mononuclear cells. The study was performed in triplicate and the results averaged.

(b) *Enumeration of B cells.* The presence of surface membrane immunoglobulins (SmIg) was used as a marker for B lymphocytes. After incubation of mononuclear cells with latex particles, a sample of cells was added to a mixture of rabbit anti-human-kappa and anti-human-lambda light chain antiserum conjugated with fluorescein (Behring Diagnostics, NJ) and incubated in an ice bath for 30 min. After washing three times, the cells were suspended in glycerin and placed on a slide. Lymphocytes were examined by ultraviolet microscopy (Zeiss RA/SN with epi-illumination) for the presence of surface fluorescence. One hundred latex-free cells were examined and the number of such cells with surface fluorescence was recorded as the percentage of B lymphocytes.

(c) *PHA stimulation.* Cultures of mononuclear cells were established in microtitre plates and incubated with various amounts of PHA (Burroughs Wellcome, Beckenham, England, purified HA17). Each culture contained 2×10^5 cells in tissue culture medium (RPMI 1640, Gibco, Grand Island, NY) with 10% FCS and antibiotics (penicillin 200 u/ml and streptomycin 200 μg/ml). Six concentrations of PHA were used: 10, 1·0, 0·5, 0·25, 0·1 and 0·01 μg/culture in order to yield a range of stimulating doses above and below 0·25 μg which had been found to be the optimal stimulating dose for normal human lymphocytes in preliminary experiments. All cultures were established in triplicate. After 66 h of incubation at 37°C, 0·5 μCi of tritiated thymidine (New England Nuclear, Boston, MA) was added and the cultures were incubated for a further 6 h. The cell cultures were harvested using an automated harvester (MASH, Microbiological Associates, Walkersville, MD) and the radioactivity of each sample was measured in a liquid scintillation spectrometer (Beckman Model LS150, Wakefield, MA). The incorporation of tritiated thymidine into DNA was expressed as c.p.m./culture.

Design of study

In vitro studies were conducted to:

(a) Investigate diurnal variation over a 24 h period (subjects 1–10 were examined at 08.00, 12.00, 20.00 and 24.00 h and subjects 11–14 at 10.00, 12.00 and 14.00 h).

(b) Establish a pre-irradiation baseline (subjects 1–10, 2 h after ingestion of psoralen and immediately prior to exposure to UVA radiation).

(c) Examine the effects of methoxsalen (0·6 mg/kg body weight) without subsequent exposure to UVA radiation (subjects 7–10, 2, 6 and 10 h after ingestion; and subjects 11–14, 2, 4 and 6 h after ingestion).

(d) Examine the effects of a single exposure to UVA radiation 2 h after ingestion of psoralen (subjects 1–10, 2, 4, 8, 14, 24, 48, 72 and 96 h after irradiation).

Analysis of results

The results were analysed by application of a set of analysis of variance and linear regression models developed for the specific design of this study. Calculations were performed using computer programmes developed for an IBM 370/168 computer.

Two types of models were used: (a) Univariate models (analysis of variance and linear regression) for analysis of data on T lymphocyte, B lymphocyte, total lymphocyte and total leukocyte counts and the response of lymphocytes to an optimal stimulating dose of PHA; and (b) a multivariate model for analysis of data on the response of lymphocytes to multiple doses of PHA. In the analysis of the response of lymphocytes to PHA, the natural logarithm of the incorporation of [^{3}H]-thymidine into DNA was used as the dependent variable; the zero value represents the mean of all results from all subjects. The models included several components of variance: subject-to-subject variability, treatment-independent variability and a residual error component. By allowing for these variations, it was possible to seek dependency of the PHA response upon the other variable, i.e., the exposure to PUVA. Several parameters of the response of lymphocytes to stimulation by graded doses of PHA were examined: the shape of the dose-response curve, the area under the curve, the magnitude of the response to all doses, and the response at the optimal stimulating dose. The response curves for graded doses of PHA were plotted and analysed visually and mathematically.

RESULTS

A diurnal variation in the total number of peripheral blood leukocytes and polymorphonuclear leukocytes was observed; both increased progressively from 08.00 to 24.00 h. For the analysis of results of *in vitro* studies performed after exposure to psoralen, with and without exposure to UVA radiation, allowance was made for this diurnal variation by normalizing results to the 08.00 h level. No diurnal variation was detected in the total number of lymphocytes, the number and percentage distribution of E-rosette forming and SmIg-positive lymphocytes, or the response of lymphocytes to stimulation with PHA.

The total and differential leukocyte counts showed no alteration following the ingestion of methoxsalen alone without subsequent exposure to radiation. However, in these circumstances a trend towards a decrease in the absolute numbers and percentage distribution of circulating E rosette-forming cells and SmIg-positive cells was detected. The mean baseline value of 1950±76 (67% of circulating lymphocytes) E rosette-forming cells decreased to 1426±196 (62%) 2 h after ingestion of methoxsalen and SmIg-positive cells decreased from 385±21 (13·3%) to 293±55 (12·7%). The absolute number and percentage distribution of null cells showed a corresponding increase. These trends were detected 2 h after ingestion and the results of the tests returned to baseline levels by 4 h after ingestion of psoralen. The incorporation of $[^3H]$-thymidine into DNA of lymphocytes following stimulation of the cells by PHA (optimal and graded doses) also tended to decrease 2 h post-ingestion and to return to baseline levels by 4–6 h. None of these trends reached significant levels ($P>0{\cdot}1$).

The four subjects who received 1 × MPD of PUVA developed minimal or no erythema (hereafter termed the minimal erythema group). The six subjects who received 2 × MPD each developed a generalized, symptomatic, 1+ to 2+ erythema with localized areas of 3+ erythema involving up to 25% of the body surface (hereafter termed the symptomatic erythema group). Post-irradiation, there was no significant alteration in the total leukocyte or the lymphocyte count in either the

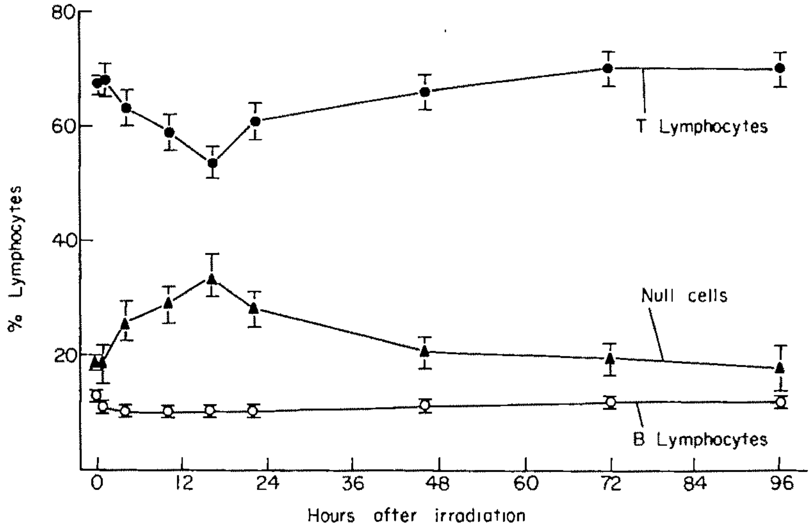

FIGURE 1. Distribution (±1 s.d.) of subpopulations of peripheral blood lymphocytes following whole-body exposure to UVA radiation after ingestion of methoxsalen. All subjects developed a symptomatic erythema.

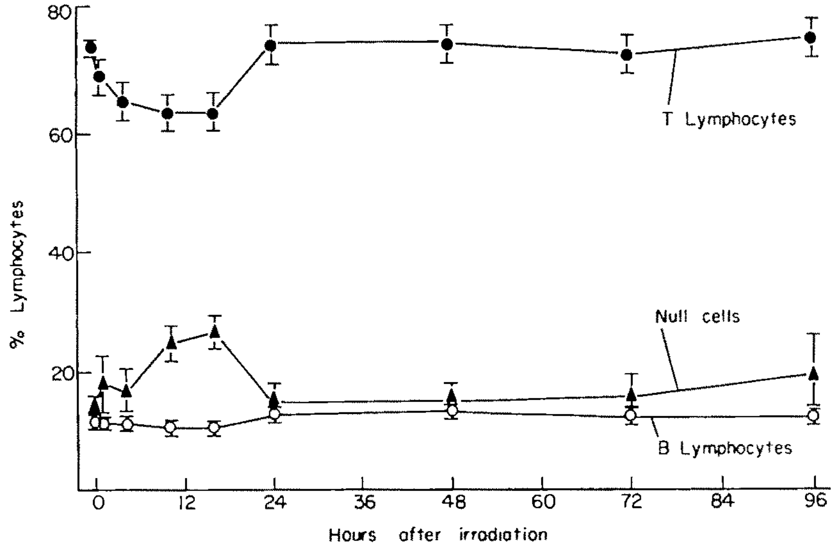

FIGURE 2. Distribution (±1 s.d.) of subpopulations of peripheral blood lymphocytes following whole-body exposure to UVA radiation after ingestion of methoxsalen. All subjects developed minimal or no erythema.

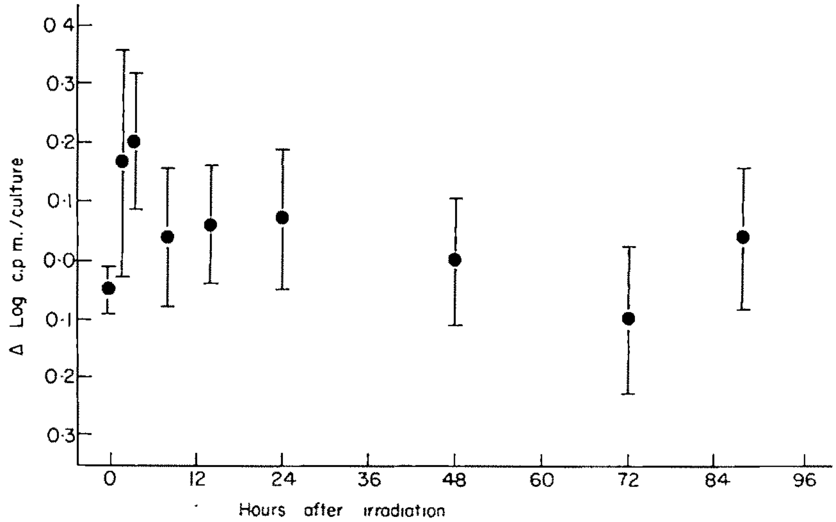

FIGURE 3. Incorporation of [^{3}H]-thymidine (±1 s.d.) by lymphocytes stimulated by PHA (all doses), expressed as difference of log c.p.m./culture from zero (zero = mean of all results from all subjects). Results are for all subjects exposed to psoralen/UVA radiation.

symptomatic or minimal erythema groups of subjects. Post-irradiation, subjects who developed a symptomatic erythema had a significant decrease in the proportion of circulating E rosette-forming cells ($P<0.001$), a significant decrease in the proportion of SmIg-positive cells ($P<0.01$) and a significant increase in the proportion of null cells ($P<0.01$). These changes were present 30 min after exposure to radiation, reached a maximum by 16 h and returned to a normal range of 48–72 h after exposure (Fig. 1). Calculation of the absolute numbers of each subpopulation of lymphocytes (from absolute lymphocyte count in peripheral blood and percentage of each subpopulation in the separated mononuclear cells) revealed trends towards a decrease in the number of E rosette-forming and SmIg-positive cells, as well as an increase in the number of null cells, but these trends were not significant. Post-irradiation, subjects who developed minimal or no erythema had a significant decrease in the proportion of circulating E rosette-forming cells ($P<0{\cdot}01$) and increase in the proportion of null cells ($P<0{\cdot}01$); the proportion of SmIg-positive cells was not significantly decreased. These changes are illustrated in Fig. 2. By comparison with Fig. 1, it can be seen that the changes tended to be less marked and of shorter duration than the changes in the symptomatic erythema group. However, the differences between the proportions of subpopulations of lymphocytes observed in the two groups of subjects were not significant.

The incorporation of tritiated thymidine into DNA of lymphocytes following stimulation by PHA (optimal and graded doses) was not significantly altered after irradiation in either the minimal or symptomatic erythema groups of subjects. An increased incorporation was seen at 2 and 4 h post-irradiation but this was not significant ($P>0{\cdot}1$). Results for all subjects are illustrated in Fig. 3. Neither the shape of the response curve to graded doses of PHA nor the area under the curve were altered following exposure to radiation.

DISCUSSION

This study demonstrated that a single whole-body exposure to UVA radiation following ingestion of methoxsalen in humans had an effect on the distribution of subpopulations of circulating lymphocytes. A dose of PUVA sufficient to produce a symptomatic erythema resulted in a decrease in the proportion of circulating E rosette-forming cells, a decrease in the proportion of SmIg-positive cells and in increase in null cells. A dose of PUVA which results in minimal or no erythema also produced a decrease in the proportion of circulating E rosette-forming cells and increase in null cells, but the proportion of SmIg-positive cells was barely altered. These alterations appeared soon after exposure to radiation, reached a maximum by 16 h and returned to baseline by 48–72 h post irradiation. The absolute numbers of each of the lymphocyte populations showed similar alterations to the percentage distributions but the changes were less significant. This difference is probably a function of the methodology employed in the study. Absolute counts are calculated from the total lymphocyte count in the peripheral blood and the percentages of each subpopulation in the separated mononuclear cells. Both determinations have their own margin of error, some mononuclear cells are lost in the separation and, while only about 5% of cells on a peripheral smear can be identified as monocytes, usually 20–30% of the separated mononuclear cells ingest latex particles. These factors probably account for the discrepancy of the results. In an earlier report (Cormane *et al.*, 1979), normal subjects showed a decreased proportion of E rosette-forming cells after four exposures to PUVA, but the proportion of SmIg-positive cells was unaltered. The use of multiple, probably sub-erythemogenic exposures may well be an important difference in methodology between that and the present study in understanding the differing effects on SmIg-positive cells.

The mechanism by which PUVA may produce an alteration in circulating lymphocytes, and the nature of that alteration, are unclear. PUVA could directly affect lymphocytes as they percolate through the skin or the effect may be indirect, via a mediator released as a result of the exposure to radiation. The temporary decrease in labelled cells (E rosette-forming and SmIg-positive) and increase in unlabelled null cells is possibly due to an alteration of surface-membrane function. Death of T and B lymphocytes could also explain the results, but then it might be expected that people repeatedly exposed to PUVA would eventually develop a deficit of these cells. Examination of patients who have been exposed to PUVA weekly for 3–5 years did not reveal any alteration of percentage or absolute numbers of circulating lymphocyte subpopulations (unpublished observations).

It is interesting to compare the results of this study with those obtained following single whole-body exposure to UVB radiation in normal human subjects (Morison *et al.*, 1979b). Erythemogenic doses of UVB radiation resulted in a similar decrease in the proportion of circulating E-rosette-forming cells and an increase in the proportion of null cells, but there was no significant alteration in the proportion of SmIg-positive cells. The changes following exposure to UVB radiation reached a maximum 8 h after irradiation, which was earlier than was obtained with PUVA; the latter produced maximal changes 16 h after irradiation. In both cases the changes appeared soon after exposure and lasted 48–72 h.

Although PUVA and UVB radiation produced similar alterations in the distribution of subpopulations of lymphocytes, other effects observed following exposure to PUVA and UVB radiation were different. Erythemogenic doses of UVB radiation resulted in a marked decrease in the response of lymphocytes to mitogen stimulation (Morison *et al.*, 1979b), whereas PUVA in erythemogenic doses had no significant effect on this response. Multiple exposures to PUVA for the treatment of psoriasis were associated with a transient decrease of the response of lymphocytes to PHA in the clearance phase of therapy (Morison *et al.*, 1979a). The design of the latter study was of course quite different from that of the present investigation; the subjects had a disease known to be associated with alterations in lymphocytes, multiple exposures were given and most exposure doses were sub-erythemogenic.

The effects of whole-body exposure to PUVA and UVB radiation differ in another important respect. Erythemogenic doses of UVB radiation produce a marked PMN leukocytosis in the peripheral blood (Morison *et al.*, 1979b). In this study it was found that exposure to PUVA, even in the presence of a marked erythemal response, had no short-term effect on the absolute numbers of circulating polymorphonuclear leukocytes. The pathogenesis of the PMN leukocytosis following exposure to UVB radiation is unknown; possibly it is due to mediators of the cutaneous inflammatory response. The mediators of PUVA and UVB-induced inflammation are probably different since it has been shown that prostaglandins are important in the early phase of a UVB-induced erythema but they do not appear to be involved in PUVA-induced erythema (Morison, Paul & Parrish, 1977; Kobza Black *et al.*, 1978). Different mediators may have differing effects on the distribution of PMN leukocytes in the body.

Exposure of bacteria to 10–50 μg/ml of methoxsalen, without subsequent exposure to UVA radiation, can cause frame-shift mutations (Clarke & Wade, 1975, Bridges & Mottershead, 1977). *In vitro* exposure of lymphocytes to 1–10 μg/ml of methoxsalen also impairs the response of these cells to PHA stimulation (unpublished observations). However, *in vivo* exposure to methoxsalen in the doses administered clinically has only been reported to alter cell function in one study (Lischka & Decker, 1978): the response of lymphocytes to a mitogen ($HgCl_2$) was found to be decreased, while the distribution of T and B lymphocytes was unaltered. Although the changes observed in the present study did not reach a level of significance, the fact that the proportion of each subpopulation of lymphocytes and the response of lymphocytes to PHA stimulation all showed an alteration soon after ingestion of psoralen supports the suggestion that the drug alone has some effect on lymphocytes.

Some important variables must be considered in investigating the effects of *in vivo* exposure to PUVA on peripheral blood lymphocytes. The dose of both drug and radiation must be considered; erythemogenic doses are most likely to induce changes in the distribution of lymphocyte subpopulations. The time of blood collection relative to the time of exposure to radiation also affects the results since changes occur early and are short-lived. Finally, the presence of disease may influence the results; for example, psoriasis has been reported to be associated with an abnormal distribution of lymphocyte subpopulations (Glinski *et al.*, 1978). These factors probably account for the variation in the results reported in different studies.

The significance of the alteration of the percentage distribution of lymphocyte subpopulations observed in this study following exposure to PUVA is unclear. The changes are short-lived and the results return to baseline values but residual damage to lymphocytes, most of which are long-lived cells, cannot be excluded. PUVA-induced alterations in lymphocytes could be beneficial or harmful. The beneficial effects of PUVA in disease states such as eczema, vitiligo and mycosis fungoides may be mediated via an effect on lymphoid cells. On the other hand, harmful effects such as the development of neoplasia or autoimmune phenomena might also result from an alteration of the distribution and function of lymphocytes.

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Some factors affecting prognosis in pemphigus vulgaris and pemphigoid

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SUMMARY

The records of twenty-eight patients dying with pemphigus vulgaris, and forty-two with pemphigoid, have been examined for factors affecting the length of their survival. Age at onset of the disease is important, old patients dying significantly more quickly than young ones. A short pre-treatment phase signifies a fulminating course in pemphigus vulgaris but not in pemphigoid.

Few general rules have been laid down for assessing which patients with bullous diseases are likely to do well with treatment. The reasons for this are simple: firstly, progress in these conditions is difficult to quantify; and secondly, they are both uncommon. In this study I have come at the subject from another angle, avoiding the first difficulty by including only patients who have died (using duration till death as a measure of the success of treatment) and the second by collecting details of all deaths from these conditions occurring in England and Wales over an 8-year period.

MATERIALS AND METHODS

The methods used to gather this information have already been described (Savin, 1979). Only undoubted cases of pemphigus vulgaris and pemphigoid, designated as such by trained dermatologists and confirmed histologically, have been included, in an attempt to delineate the differences between the two conditions. Full details were available for twenty-eight patients with undoubted pemphigus vulgaris and forty-two with pemphigoid.

RESULTS

Length of survival after starting systemic therapy

Figures 1 and 2 show the percentage surviving after the start of steroid treatment plotted with respect to time. About half of the pemphigus patients who died did so within 12 weeks, but thereafter the death rate slowed markedly, and 36% were still alive 1 year after treatment had been started. In contrast, nearly three-quarters of the pemphigoid patients who died did so within 12 weeks and only 10% were still alive after 1 year.

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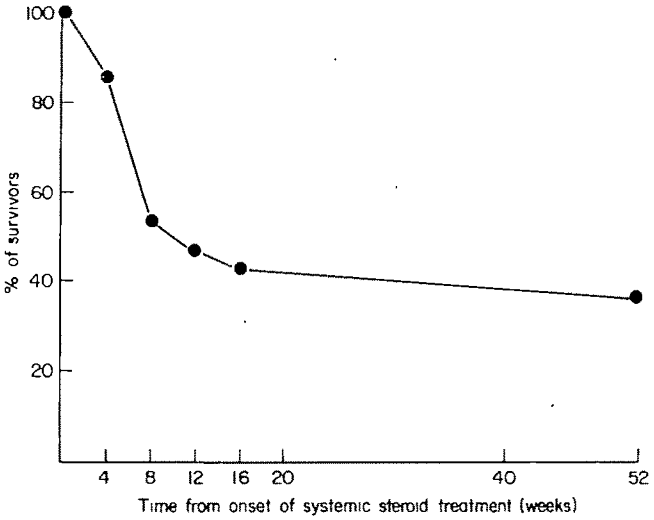

FIGURE 1. The percentage of patients surviving after the start of systemic steroid therapy plotted with respect to time. Data from twenty-eight patients with pemphigus vulgaris.

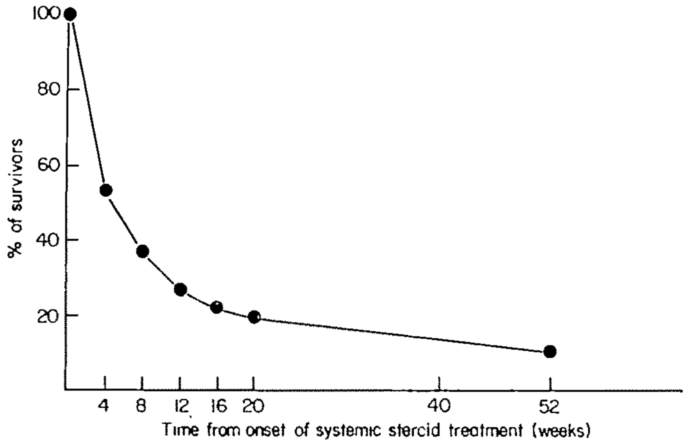

FIGURE 2. The percentage of patients surviving after the start of systemic steroid therapy plotted with respect to time. Data from forty-two pemphigoid patients.

Age

The mean age at onset of the pemphigus patients dying within 12 weeks of the start of treatment was 75·3 years, in sharp contrsst to the 55·5 years of those surviving the first twelve weeks of treatment (significant at the 1% level, Wilcoxon ranking test). Figure 3 shows the length of survival after treatment of these patients plotted against their age at the onset of the disease. The slope of the regression line indicates a strongly significant tendency for the old patients who die to do so more quickly than the young ones ($t=3{\cdot}7$, $P<0{\cdot}01$).

The mean ages at onset of the pemphigoid patients dying before and after 12 weeks of treatment (76·2 and 65·0 years) were also significantly different at the 1% level (Wilcoxon ranking test). Figure 4 shows length of survival plotted against age at onset of the disease: as with the pemphigus vulgaris patients, the slope of the regression line shows a significant tendency for old patients to die especially quickly ($t=2{\cdot}4$, $P<0{\cdot}05$).

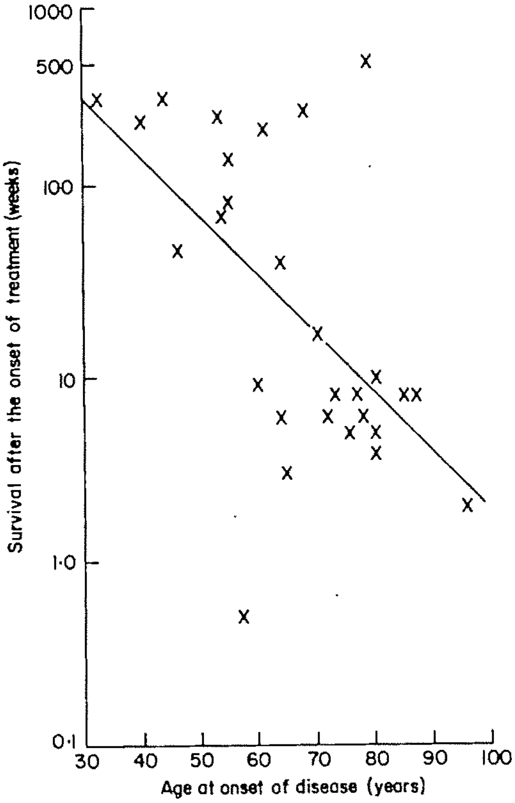

FIGURE 3. The length of survival after the onset of steroid therapy (logarithmic scale) plotted with respect to age at the onset of the disease. Data from twenty-eight patients with pemphigus vulgaris.

Duration of symptoms before treatment

Two of the pemphigus patients had genuine symptoms for as long as 1 year before the onset of treatment, but nearly 80% had had symptoms for less than 12 weeks. There was no significant relationship between age at onset and the duration of symptoms before treatment. However, those with a short pre-treatment phase tended to survive poorly (Table 1).

The older pemphigoid patients, in contrast, tended to have long pre-treatment phases, but there was no relationship between the length of the pre-treatment phase and survival after the start of systemic therapy.

Steroid dosage

Many patients were treated with a rapid succession of different corticosteroid preparations, often supplemented by A.C.T.H. For this reason it was not possible to form any impression of the superiority of one preparation over another. For simplicity, doses are expressed here as their equivalents in mg of prednisolone. All patients had received systemic steroids, but, dying as they did between 1962 and 1969, none had been given methotrexate.

Two convenient measures of steroid dosage are the starting dose and the highest dose used. The mean doses of the pemphigus patients dying within 12 weeks of the start of treatment (starting dose 49·1 mg and highest dose 109·1 mg prednisolone equivalents) did not differ significantly from those of the patients surviving 12 weeks (35·0 and 94·3 mg).

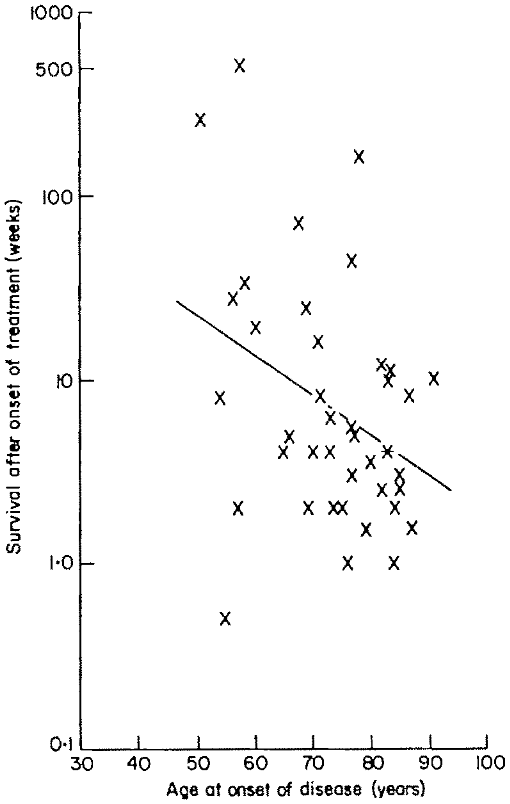

FIGURE 4. The length of survival after the onset of systemic steroid therapy (logarithmic scale) plotted with respect to age at the onset of disease. Data from forty-two patients with pemphigoid.

TABLE I. Relationship between pre- and post-treatment phases of twenty-eight patients with pemphigus vulgaris

Duration from onset of disease until start of steroid treatment (weeks)	No. and percentage of patients surviving more than 12 weeks after start of systemic steroids
0–4 (eleven patients)	2 (18%)
5 or more (seventeen patients)	11 (65%)

The comparable figures for the pemphigoid patients, 37·7 and 70·2 mg for those surviving less than 12 weeks and 49·1 and 69·5 mg for those surviving more than 12 weeks, were also not significantly different. In neither condition were the selected steroid doses related to the patients' age at the onset of their disease.

Sex and the site of the initial lesions

These factors were not significantly related to survival although the pemphigus patients whose first lesions were in the mouth had slightly longer pre- and post-treatment phases.

DISCUSSION

The clinical course of patients dying with bullous diseases can be divided into two phases. The first is from the onset of the disease until systemic treatment is given; the second is from the start of this treatment until death.

The first phase represents to some extent the speed of attack of the disease in that the rapid onset of extensive blistering is likely to call for the use of systemic treatment sooner than does a slow moving sparse eruption. Obviously many other factors are involved such as threshold for complaint, ease of access to medical help, and the whim of the dermatologist—however the length of the first phase may be a rough guide to the tempo of the disease process.

Lever (Lever & White, 1963) has always stressed that the earlier treatment is started, the more easily can pemphigus be controlled. At first sight this does not seem to have been borne out by the present study, in which pemphigus vulgaris patients treated with systemic steroids soon after the onset of their disease tended to die more quickly than those whose treatment started later. The findings of Sanders, Brodey & Nelson (1960) were similar: of their twenty-four patients whose corticosteroid treatment began within 6 months, fifteen died as a direct result of the pemphigus. In contrast, only two of their thirteen patients who had received no systemic treatment for the first 6 months, died from the disease. They argued that their data did not support the assumption that early treatment decreases mortality. Ryan (1971) also found that prompt vigorous initial treatment did not materially influence the mortality rate. However, these differing views about the need for early treatment are based on a misunderstanding.

Lever had used the word 'early' to describe patients whose skin showed only mild localized involvement and contrasted their progress with that of patients with extensive skin lesions (Lever 1965). In the present study, and that of Sanders *et al.* (1960), on the other hand, 'early' means soon after the onset of the disease, taking no account of its extent. Other workers (Asboe-Hansen, 1970; Fernandez, Dharani & Desai, 1970) have also found that pemphigus vulgaris with a sudden fulminating onset often follows a highly malignant course, and this seems the obvious explanation of the present results; the severest cases progressing rapidly and quickly requiring treatment to control them. In contrast, no hint of a fulminating type of pemphigoid has been found in the present study. The long pre-treatment phase of the old patients does not necessarily imply a slower course, although this is possible, as in this age group symptoms may be less well remembered and less quickly acted on.

Age at onset has long been felt to be important in determining survival in pemphigus. This seems to have been so even before systemic steroid therapy was available, but these early reports are hard to interpret as patients with pemphigus and pemphigoid were grouped together. The same trend can now be detected in publications dealing with methotrexate therapy (Jablonska, Chorzelski & Blaszczyk, 1970) but the relationship between age and mortality is best documented in the literature dealing with systemic steroid therapy. A high proportion of old patients with pemphigus are known to die despite systemic treatment (Ryan, 1971; Krain, 1974). The present study sheds further light on this topic. It can provide no further information about the ratio of patients dying and surviving in the different age groups, as only patients who died have been included. However, one of the most striking findings has been the contrast between the short survival of the old patients and the much longer survival of the young ones. This has not been described before. It seems therefore that old patients with pemphigus or pemphigoid die more quickly as well as more frequently than the young ones. This poor survival of old patients is not a feature unique to these bullous diseases: it has been well documented in many serious conditions including renal failure (Henari *et al.*, 1977), appendicitis (Peltokallio & Jauhiainen, 1970) and haematemesis (Schiller, Truelove & Williams, 1970).

One striking difference between the patients dying with pemphigus and pemphigoid is the high proportion, about 90%, of those with the latter who do so within 1 year. However, it does not follow from this that pemphigus vulgaris is more benign than pemphigoid; the opposite is known to be the case. Several circumstances have combined to produce this apparent paradox. If a disease lasts only a short time, deaths from it must occur soon after treatment has started. Pemphigoid has a shorter course, with about 30% of patients needing treatment for less than one year (Stevenson, 1960), while pemphigus vulgaris usually lasts indefinitely. Deaths from pemphigoid will therefore be packed into the early months when the largest number are at risk. Pemphigoid patients also need much lower doses of steroids to control their symptoms. and the late side effects, some of which may be fatal, may never develop. Finally, pemphigoid patients fall particularly into the older age groups and so tend to die quickly after treatment is started, the younger patients, particularly with pemphigus, surviving long enough to experience the fatal cumulative effects of high and prolonged steroid doses (Savin, 1979).

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Epidemiology of congenital pigmented naevi: II. Risk factors

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SUMMARY

Genetic and environmental risk factors were investigated in a sample of 989 non-malformed newborn infants with skin pigmented naevi (PN), and in 989 non-malformed non-PN, control babies. The samples were obtained from a population of 531,831 livebirths, from fifty-nine South-American maternity hospitals. No significant differences were observed between the PN and control groups for the following risk factors: sex, twinning, parental consanguinity, socioeconomic level, parental ages, birth order, fetal presentation, type of delivery, postnatal mortality, and first trimester of pregnancy histories for maternal chronic illnesses or immunizations, radiation exposure, drug intake, and vaginal bleeding. PN infants were associated with high frequencies of Black racial ancestry, positive prenatal history for maternal acute illnesses, and high mean values for length of gestation and birth weight. The observed association of PN with maternal acute illnesses could be a spurious one, due to partial dependence upon Black racial ancestry, and to maternal memory bias. The analysis of the intrauterine growth curves showed that PN infants have a low prematurity rate, and a high mean birth weight within the postmature group, lacking the normal post-term weight loss pattern shown by the control newborn group. This observation suggests that congenital PN appear in the latest stages of intrauterine development. No evidence was obtained to allow us to consider congenital PN as true developmental anomalies.

The pathological significance of pigmented naevi (PN) present at birth remains obscure. Information about their aetiology and pathogenesis includes no more than a few familial (Goodman *et al.*, 1971) and twin (Cantu *et al.*, 1973) observations concerning the most severe forms of giant PN. The prognostic meaning of the newborn's PN in terms of future evolution of the PN itself, risks of malignancy, or development of other generalized syndromes, of which the skin PN could be the earliest clinical manifestation, is difficult to evaluate due to the lack of relevant information. While the most direct and effective way to solve this problem would be through a long term longitudinal cohort study of a large series of newborn infants presenting with PN and of a suitable control group, prospective

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studies are discouragingly expensive and difficult to perform. These facts account for the lack of prognostic data for congenital PN.

Meanwhile, some knowledge may be obtained through a different approach, as with the correlation studies (Leck, 1978) involving genetic and environmental risk factors known to be associated with developmental anomalies. The underlying working hypothesis of the present investigation was that the congenital PN are a true developmental anomaly (anomaly of embryological development) and that a correlation should be found between this condition and some variables of direct or indirect aetiological significance (risk factors).

MATERIALS AND METHODS

This study involved 989 non-malformed newborn infants with pigmented naevi (PN) of the skin, detected during the physical examination of 531,831 livebirths from fifty-nine maternity hospitals participating in the Latin American Collaborative Study of Congenital Malformations: E.C.L.A.M.C. (Castilla *et al.*, 1973) in the 1967–1976 period. The morphological description of the PN included in this paper, as well as other methodological details, have already been published (Castilla, Dutra & Orioli-Parreiras, 1981).

A control group was obtained from the same livebirth population by selecting non-malformed newborn infants of the same sex, born next to the birth of a PN infant in the same hospital. Therefore, there was a one-to-one case-control matching by sex, place and time of birth. Babies in the PN and control groups did not have any detected congenital anomaly.

A special form, containing family, prenatal, and perinatal history data, was filled in for each PN and control newborn infant. Family data included: racial background, parental consanguinity, socioeconomic level (based on an eight point score according to parental education and occupation), parental ages, and birth order. Even though information was also collected on the familial incidence of congenital PN, it was excluded from analysis because the congenital nature of the PN described in the relatives was not considered to be reliable. Prenatal data included: length of gestation, birth weight, and events of the first trimester of pregnancy, namely, maternal immunizations, acute and chronic illnesses, radiation exposure, drug intake, and vaginal bleeding. Perinatal data consisted of fetal presentation, type of delivery, twinning, sex, and mortality within the first 3 days of life.

The frequency distributions for all those variables in the PN and control groups were compared, and differences were tested by the usual statistical significance tests: Student's '*t*'-test for parental ages, length of gestation, and birth weight; Kolmogorov–Smirnov's test for birth order and socioeconomic level; and chi-square contingency test for all the other variables. Since controls were matched by sex, the sex ratio of the PN group was compared with that of the total livebirth population (males/females: 270,937/260,894 = 0·51).

RESULTS

Newborn infants with pigmented naevi did not differ from the unaffected control babies in the frequency of twinning, parental consanguinity, fetal presentation, type of delivery, mortality within the first 3 days of life, birth order, or parental ages. Nor were differences observed in sex ratio and socioeconomic level (Table 1).

There were twelve pairs of twins within the PN group, seven concordant and five discordant for sex. All pairs included only one member affected with PN. Due to the high degree of racial admixture in Latin-American populations, race was not considered as an attribute of the individual but as the participation of each racial group to the ethnic background of each newborn infant. This was achieved

TABLE 1. Compared frequency distributions for several risk factors in newborn infants with pigmented naevi, and in matched controls

Risk factors	Naevi group		Control group		
	n	%	*n*	%	*P*
Male sex*	493/989	49·9			—
Twinning	12/988	1·2	8/988	0·8	—
Parental consanguinity	5/978	0·5	4/983	0·4	—
Race†					
Native	745/904	82·4	742/904	82·1	—
Latin-European	416/904	46·0	419/904	46·4	—
Black	92/904	10·2	61/904	6·8	+
Non-Latin-European	34/904	3·8	44/904	4·9	—
Arabian	18/904	2·0	17/904	1·9	—
Oriental	4/904	0·4	7/904	0·8	—
Jews	2/904	0·2	3/904	0·3	—
First born	303/986	30·7	319/987	32·2	—
FTP‡					
Immunizations	19/980	1·9	9/985	0·9	—
Acute illnesses	71/975	7·3	47/984	4·8	+
Chronic illnesses	34/979	3·5	34/983	3·5	—
Radiation	6/980	0·6	6/986	0·6	—
Drugs	199/978	20·4	170/982	17·3	—
Vaginal bleeding	29/973	3·0	28/985	2·8	—
Abnormal fetal presentation	31/978	3·2	35/977	3·6	—
Caesarean section delivery	96/978	9·8	102/977	10·4	—
Death up till three days of age	4/959	0·4	4/963	0·4	—
Socioeconomic level: $\bar{x}$	3·02		3·10		—
Birth order: $\bar{x}$	3·06		2·90		
Maternal age (years): $\bar{x}\pm$s.e.	25·55±0·20		25·50±0·21		—
Paternal age (years): $\bar{x}\pm$s.e.	29·62±0·25		29·11±0·24		—
Gestation age (weeks): $\bar{x}\pm$s.e.	39·48±0·09		39·23±0·09		+
Birth weight (hg): $\bar{x}\pm$s.e.	32·95±0·16		32·07±0·17		+++

n: Number of cases with the risk factor/total number of cases with specified data.

P: Significance of differences between the naevi and control groups. —($P>0·05$): not significant, +($P<0·05$) and +++($P<0·001$): significant.

* Compared against total livebirth population: Males: 51·0%.

† Participation of each racial group in the ethnic background.

‡ First trimester of pregnancy events.

by recording all racial groups that could be identified in the infant's ancestry. Information on race was available for only 904 of the 989 babies of the two groups, PN and controls, because this item was not introduced into the study until 1970. Seven racial groups were considered: Natives (defined as, most probably, an admixture of American-Indians with Latin-Europeans), Latin-Europeans, African negroes (Blacks), Non-Latin-Europeans, Arabs (mainly from Syria and Lebanon), Orientals, and Jews. The only significant difference observed was a higher frequency of Black ancestry in the PN (10·2%) than in the control group (6·8%).

No differences were seen for maternal immunizations, maternal chronic illnesses, radiation exposure, drug intake, or vaginal bleeding within the first trimester of pregnancy between the PN and control infants. The PN group had a higher frequency of maternal acute illnesses (7·3%) than the control group (4·8%). This difference was not based on any particular maternal acute illness, the most common one within both groups being influenza.

The average length of gestation, measured in weeks between the first day of the last normal menstrual period to the birthdate, was longer in the PN group ($\bar{x}$: 39·48; s.e.: 0·09) than in controls ($\bar{x}$: 39·23; s.e.: 0·09). The birth weight, measured in hectograms ,was also higher in the PN ($\bar{x}$: 32·95; s.e.: 0·16) than in the control ($\bar{x}$: 32·07; s.e.: 0·17) groups.

When the intrauterine growth, expressed as the average birth weight for each weekly interval of gestation age, was compared between the PN and the control groups, it was observed that the weight-increase curves were similar until week 40 of pregnancy. Thereafter, while the control curve showed the well known postmature weight-loss pattern, the PN curve continued its weight-gain linear trend (Fig. 1).

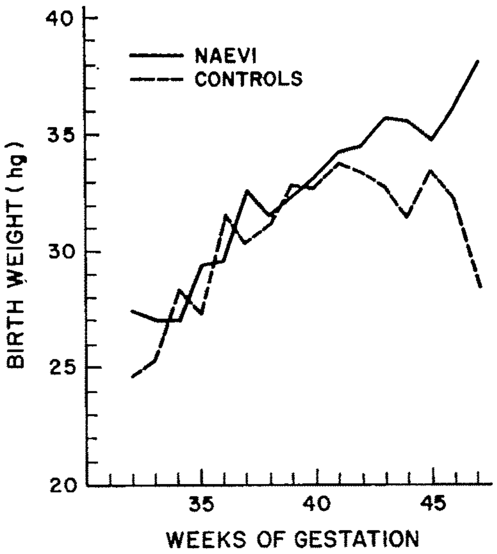

FIGURE 1. Intrauterine growth of pigmented naevi and control newborn infants.

The lower part of the curve (<37 weeks) included a lower proportion of cases in the PN group (62/823 = 7·5%), as compared with the control group (86/825 = 10·4%) (χ_1^2: 3·87; $P<0·05$). However, within this class of premature births, the frequency of babies weighing 25 hg or less in the PN (17/62 = 27·4%) and in the control (29/86 = 33·7%) groups was not significantly different. Therefore, the PN newborn infants had a lower than expected prematurity rate even though within the premature class their birth weights were similar to those of the controls.

The upper part of the curve (>39 weeks) included similar proportions of cases in the PN (423/823 = 51·5%) and in the control (392/825 = 47·5%) groups. Nevertheless, within this class of postmature births, the frequency of babies weighing 33 hg or more was significantly higher in the PN (266/423 = 62·9%) than in the control (193/392 = 49·2%) group (χ_1^2: 14·86; $P<0·001$). Therefore, even though the postmaturity rate of the PN newborn infants did not differ from control values, they were heavier than expected for this postmature class of births.

The proportion of postmature heavy newborn infants (>39 weeks and 32 hg) was significantly higher within the PN group (32·3%) than among the controls (23·4%) (Table 2). No correlation

TABLE 2. Frequencies of heavy (33≥ hg) postmature (40≥ weeks) infants according to their Black racial ancestry, and the prenatal history of acute maternal illnesses, in the pigmented naevi and in matched control groups

				Heavy postmature				
				Yes			Differences	
	Groups			%	*n*	Total *n*	Groups	*P**
1	Naevi:	Blacks:	Acute I.:	40·0	4	10	1–2	—
2			No acute I.:	32·9	24	73	1–8	—
3			Total:	37·7	28	83	2–9	—
4		Non Blacks:	Acute I.:	32·1	17	53	3–6	—
5			No acute I.:	32·2	221	687	3–10	—
6			Total	32·2	238	740	4–5	—
7		Total:	Total:	32·3	266	823	4–11	—
8	Controls:	Blacks:	Acute I.:	16·7	1	6	5–12	+++
9			No acute I.:	21·7	10	46	6–13	+++
10			Total:	21·2	11	52	8–9	—
11		Non Blacks:	Acute I.:	20·7	6	29	10–13	—
12			No acute I.:	23·7	176	744	11–12	—
13			Total:	23·5	182	773	7–14	+++
14		Total:	Total:	23·4	193	825		

* Significance of differences between groups. —($P>0{\cdot}05$): not significant, +++ ($P<0{\cdot}001$): significant.

could be proved between heavy post-maturity and either Black racial ancestry or maternal acute illness. The higher proportion of postmature heavy infants in the PN than in the control group stood out even after removal of the cases with Negro background and/or prenatal acute illnesses.

Within the control group, the frequency of acute illnesses in the first trimester of pregnancy was significantly higher in the Black (11·5%) than in the non-Black (3·9%) ancestry classes (Table 3). This positive correlation could not be proved for the PN group, where the frequency of acute illnesses was not significantly different in the Black (12·0%) and in the non-Black (7·2%) racial classes. The

TABLE 3. Frequency of prenatal history of acute maternal illness, in infants with pigmented naevi and in controls, according to their Black racial background

			Acute illnesses				
			Yes			Differences	
			%	*n*	Total *n*	Groups	*P*
1	Naevi:	Blacks:	12·0	10	83	1–2	—
2		Non Blacks:	7·2	53	740	4–5	+
3		Total:	7·6	63	823	1–4	—
4	Controls:	Blacks:	11·5	6	52	2–5	++
5		Non Blacks:	3·9	29	773	3–6	++
6		Total:	4·2	35	825		

* Significance of differences between groups. —($P>0{\cdot}05$): not significant, +($P<0{\cdot}05$) and ++ ($P<0{\cdot}01$): significant.

higher frequency of a positive prenatal history for acute illnesses in the PN (7·2%) than in controls (3·9%) remained significant when only the non-Black classes of both groups were compared. Consequently, the higher frequency of acute illnesses in the first trimester of pregnancy of the PN newborn infants may be only partially secondary to their greater Black racial extraction.

DISCUSSION

The higher frequency of Black racial admixture in infants with pigmented naevi (PN) found in this study reinforces the concept of a higher risk to develop PN prenatally for the black than for the white coloured races, as has been suggested previously by the data of Pratt (1953) and of Walton, Jacobs & Cox (1976) from U.S.A. White and Black newborn infants.

In Latin-American countries, where the population with black racial admixture usually belongs to the lower socioeconomic classes, this race-socioeconomic correlation may interact as compound variables, thus creating false positive correlations between race-dependent diagnoses and socioeconomic-dependent risk factors. Such a situation had already been identified within the material presented here for a false positive correlation between polydactyly and low birth weight, where the low birth weight was secondary to low socioeconomic level, which was associated with high Black racial admixture; polydactyly is known to be more frequent in Black than in any other ethnic group (Passos *et al.*, 1978). Since PN have a higher frequency in Blacks, similar effects through socioeconomic differences are to be expected. Such differences may be subtle enough to be undetected by the rough measurement of socioeconomic status used here. Furthermore, the identification of racial ancestry in highly mixed populations does not have the precision of individual race assignment applied in racially stratified populations.

The high incidence of a positive prenatal history of maternal acute illnesses cannot be considered important information because of its partial correlation with black race, which may be due to low socioeconomic status. In addition, prenatal history is very susceptible to maternal memory bias in retrospective case-control study.

The elevated birth weight found in the PN group is the most relevant positive result of this study. First of all, the observed difference between the PN and control groups is great enough to rule out the possibility of a casual spurious finding. Secondly, birth weight and length of gestation are objective variables. Thirdly, the interacting effects of racial differences are expected to act in an opposite direction, that is, reducing the mean birth weight of PN infants. Therefore, it may be suggested that the correlation between PN and high birth weight could actually be even greater than observed.

The difference in intrauterine growth patterns observed between PN and control infants could be due to a lower frequency of PN in immature newborn infants. This would then be the basis for the low rate of prematurity as well as for the high mean birth weights in the postmature group of PN infants. A lower frequency of PN in immature newborn infants suggests that PN become clinically visible in the later stages of pregnancy. Pigmented naevi are known to appear at different ages from birth to adult life, mostly around puberty (Lewis & Johnson, 1968). The present data indicate that this continuous process, known to start before birth, could have a spurt in late prenatal life, similar to the pubertal one.

This study of risk factors associated with PN, aimed at obtaining epidemiological evidence supporting PN as developmental anomalies, produced negative results in this respect.

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Incorporation of D-(^{3}H)glucosamine into normal and psoriatic epidermal glycoconjugates

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SUMMARY

Samples of normal, uninvolved and involved psoriatic skin were maintained for 18 h in organ culture in the presence of D-(6-^{3}H)glucosamine which is a precursor of cell-surface associated carbohydrates in the epidermis.

The total incorporation into involved psoriatic epidermis (solubilized with 8 mol/l urea, 5% sodium dodecyl sulphate, 10 mmol 2-mercaptoethanol) was less than into normal or uninvolved epidermis. This decrease was found whether specific radioactivities were expressed in terms of area, wet weight or protein content of the epidermis.

Electrophoresis revealed that the major labelled component in normal and uninvolved epidermis had a high molecular weight. The labelling of this material was significantly reduced in involved psoriatic epidermis. Using pig epidermis we have shown previously that this material represents mainly extracellular glycosaminoglycans and proteoglycans. The decreased labelling (and presumably synthesis) of these extracellular carbohydrates may be related to the failure of mechanisms controlling cell proliferation and to the altered cell interactions which are found in psoriatic epidermis.

Electrophoresis also showed that a non-glycosylated protein, molecular weight approximately 50,000, which was present as a single band in both normal and uninvolved epidermis was always present as a doublet band in the involved psoriatic epidermis.

Histochemical studies have indicated that the glycoconjugates present in the cell surface carbohydrate coat or 'glycocalyx' (Mercer, Jahn & Maibach, 1968; Wolff & Schreiner, 1968; Fritsch, Wolff & Hönigsmann, 1975) are abnormal in psoriatic epidermis (Mercer & Maibach, 1968; Orfanos *et al.*, 1973; Orfanos, Mahrle & Runne, 1976). The staining of the 'glycocalyx' appeared to be reduced in the living cell layers and increased in the horny cell layers. These abnormalities of surface glycoconjugates may be involved in the failure of mechanisms controlling cell proliferation, in the alteration of cell-cell adhesion and in the defective desquamation all of which are characteristic of the epidermis in psoriasis (Mahrle & Orfanos, 1977; Skerrow, 1978).

We have been investigating the structure and synthesis of the glycoconjugates present at the

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epidermal cell surface. Using the radioactive precursors L-(^{3}H)fucose and D-(^{3}H)glucosamine together with sodium dodecyl sulphate polyacrylamide gel electrophoresis we have shown that two distinct types of surface-associated glycoconjugate are synthesized in the epidermis of cultured pig skin slices (King, Tabiowo & Williams, 1980). Plasma membrane glycoproteins which are strongly anchored in the membrane were labelled to a similar extent by both precursors. In addition a high molecular weight component was heavily labelled by D-(^{3}H)glucosamine but not at all by L-(^{3}H)-fucose. This material was considered to consist of glycosaminoglycans and proteoglycans since it was also labelled by (^{14}C)glucuronate and to a lesser extent by $^{35}SO_4^{2-}$ and (^{14}C)amino acids. Further it was completely degraded to low molecular weight components by testicular hyaluronidase yet it resisted digestion with proteases. This material was preferentially released when the epidermal cells were dispersed with trypsin and was therefore associated mainly with the external surfaces of the cells. However it was not present in crude or purified plasma membranes isolated from the epidermis by sucrose density gradient centrifugation. It was therefore either only weakly bound at the outer cell surface or it was present in the intercellular spaces. We have described this material as extracellular.

In a previous autoradiographic study it was shown that the distribution of L-(^{3}H)fucose labelled glycoproteins was altered in psoriatic epidermis (Mann, Williams & Gray, 1980). A smaller proportion of the L-(^{3}H)fucose labelled glycoprotein was present at the surface of the psoriatic cells. In the present electrophoretic study of surface glycoconjugates in psoriasis we have used D-(^{3}H)glucosamine rather than L-(^{3}H)fucose for two reasons. Firstly, D-(^{3}H)glucosamine gives higher levels of labelling than comparable amounts of L-(^{3}H)fucose and is therefore more sensitive for biochemical studies. Secondly, D-(^{3}H)glucosamine labels extracellular as well as plasma membrane bound components and is therefore a wider range probe for investigating surface carbohydrate changes.

MATERIALS AND METHODS

Skin biopsies

Specimens of skin from uninvolved and plaque areas were taken freehand from volunteer psoriasis patients using 1% lignocaine. Specimens of normal skin were taken either from healthy volunteers using the same procedure or from skin removed during surgical procedures. In the latter case a Castroviejo keratotome set to cut at 0·2 mm was used.

Labelling with D-(^{3}*H*)*glucosamine*

The skin slices containing all the epidermis together with some underlying dermis were trimmed to 0·7 cm × 0·7 cm square on graph paper. Each slice was then floated on 1 ml of Eagles minimum essential growth medium buffered with 20 mmol HEPES pH 7·3 and containing 10% fetal calf serum, Fungizone and gentamicin (5 μg/ml of each) and 10 μCi/ml of D-(^{3}H)glucosamine hydrochloride (>10,000 Ci/mol; Radiochemical Centre, Amersham, U.K.) in a 3 cm vented plastic petri dish. The dishes were placed in a sealed container and were incubated at 37°C for 18 h. Each slice was washed six times with 10 ml of phosphate buffered saline, was floated on 1 ml of 1 mol/l $CaCl_2$ at 37°C for 1 h and the epidermis was peeled away from the dermis. The epidermis was blotted dry and was weighed.

Solubilization of epidermis

The epidermis was homogenized in 0·25 ml of 8 mol/l urea, 5% sodium dodecyl sulphate using a small hand-held Potter type homogenizer with ground glass surfaces. 2-Mercaptoethanol was added to a final concentration of 10 mmol and the homogenate was heated for 10 min in a boiling water bath. After cooling to room temperature, the homogenate was centrifuged for 30 min at 12,000 *g* and at room temperature. The supernatant was removed and the pellet consisting of broken horny cells together with tonofilament aggregates was suspended in 8 mol/l urea, 5% sodium dodecyl sulphate, 10 mmol 2-mercaptoethanol (urea/SDS/ME). The protein content of the supernatant and pellet was measured by the Hartree (1972) modification of the method of Lowry *et al.*, (1951) using bovine serum albumin as standard. Radioactivity in the supernatant and pellet was measured by liquid scintillation counting with 5 ml of PCS scintillation fluid. The counting efficiency was about 35%.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Samples of the urea/SDS/ME extracts containing known amounts of protein and radioactivity were analysed by electrophoresis in 7% polyacrylamide gels using the discontinuous buffer system of Ugel, Chrambach & Rodbard (1971) as described previously (King *et al.*, 1980). Wherever possible samples of normal, uninvolved and involved epidermis were analysed in the same electrophoresis run. Gels were allowed to stand overnight in 10% trichloroacetic acid and were washed a further three times (1 h each wash) in 10% trichloroacetic acid. The gels were then frozen with solid CO_2 and were sliced into 2 mm sections using a Bio-Rad Model 190 gel slicer. The gel slices were then dissolved in a minimum (two or three aliquots of 0·5 ml) of H_2O_2 (100 vols)/conc NH_3 (95:5 by vol) at about 60°C and the radioactivity in each dissolved gel slice was measured using 5 ml of PCS scintillation fluid with a counting efficiency of about 30%. Parallel gels were stained for protein with Coomassie brilliant blue.

RESULTS

Treatment with 8 mol/l urea, 5% sodium dodecyl sulphate, 10 mmol 2-mercaptoethanol at 100°C solubilized 85% of the total D-(^{3}H)glucosamine incorporated into normal and uninvolved epidermis and 64% of the total protein (Table 1). The total amount both of D-(^{3}H)glucosamine and of protein extracted from normal and uninvolved epidermis was also similar. Compared to normal and uninvolved epidermis, 8 mol/l urea, 5% sodium dodecyl sulphate, 10 mmol 2-mercaptoethanol solubilized a smaller proportion both of the D-(^{3}H)glucosamine (75%) and of the total protein (49%) in psoriatic epidermis. The total amount of D-(^{3}H)glucosamine labelled material in psoriatic epidermis was usually lower than in the controls despite the fact that increased amounts of protein were solubilized.

Because of the marked morphological differences between normal and psoriatic epidermis, the incorporation of D-(^{3}H)glucosamine was expressed using three data bases: area of epidermis, wet

TABLE 1. Extraction of radioactivity and protein from D-(^{3}H)glucosamine labelled epidermis

	Wet weight of biopsy (mg)	C.p.m. × 10^{-3} extracted by urea/SDS/ME	Extracted c.p.m. as % of total	Protein extracted by urea/SDS/ME (mg)	Extracted protein as % of total
Normal (12)*	7·3 ± 0·6	227 ± 64	85 ± 2	1·0 ± 0·18	64 ± 2
Uninvolved (15)†	7·1 ± 0·7	230 ± 57	85 ± 3	1·14 ± 0·15	64 ± 2
Involved (15)	16·6 ± 1·7	173 ± 30	75 ± 4	1·41 ± 0·15	49 ± 4

* Figures in brackets denote number of samples.

† Mean ± standard error of mean.

TABLE 2. Specific radioactivity* of D-(^{3}H)glucosamine labelled epidermis expressed in terms of area, weight and protein content

	Area	Weight	Protein
	Radioactivity/mm^2† of epidermis (c.p.m.)	Radioactivity/μg wet weight of epidermis (c.p.m.)	Radioactivity/μg of extracted protein (c.p.m.)
Normal	4622±1300	37±11·6	276±87
Uninvolved	4693±1159	31±4·5	243±81
Involved	3539±619	13±2·8	143±47

* Calculated from the data in Table 1.

† Radioactivity refers to the radioactivity extracted by 8 mol/l urea, 5% sodium dodecyl sulphate, 10 mmol 2-mercaptoethanol.

TABLE 3. Incorporation of D-(^{3}H)glucosamine into the high molecular weight component in normal and psoriatic epidermis

	Total radioactivity in high molecular weight component in biopsy (c.p.m. × 10^{-3})
Normal (7)	68±15
Uninvolved (9)	59±18
Involved (9)	20±8
Involved vs normal*	$P<0{\cdot}01$
Involved vs uninvolved	$P<0{\cdot}05$
Uninvolved vs normal	ns†

* Student's '*t*'-test.

† Not significant.

weight of epidermis and mg of epidermal protein (Table 2). Whichever data base was used the specific radioactivity of the involved psoriatic epidermis was less than that of both uninvolved and normal epidermis. There was a wide variation in the labelling of specimens cultured over the time period in which these experiments were carried out. However, the labelling of the involved epidermis was lower than the uninvolved in eleven out of fifteen (area), fifteen out of fifteen (weight) and thirteen out of fifteen (protein) cases when samples from the same psoriatic patient were cultured at the same time.

To determine whether this decrease in the total labelling was reflected in the incorporation of D-(^{3}H)glucosamine into individual components samples of the solubilized epidermis were analysed by electrophoresis in 7% polyacrylamide gels. The gels were washed in 10% trichloroacetic acid to remove low molecular weight, acid-soluble material, were sliced and the radioactivity in each gel slice was measured. The total radioactivity recovered off the washed gel accounted for 59%, 61% and 46% of the applied radioactivity from normal, uninvolved and involved epidermis. Fig. 1 compares the radioactivity profiles obtained in two typical experiments. As we found previously with pig epidermis (King *et al.*, 1980) the major component labelled with D-(^{3}H)glucosamine remained close to the top of the gel indicating a high molecular weight. This component was present in similar amounts in

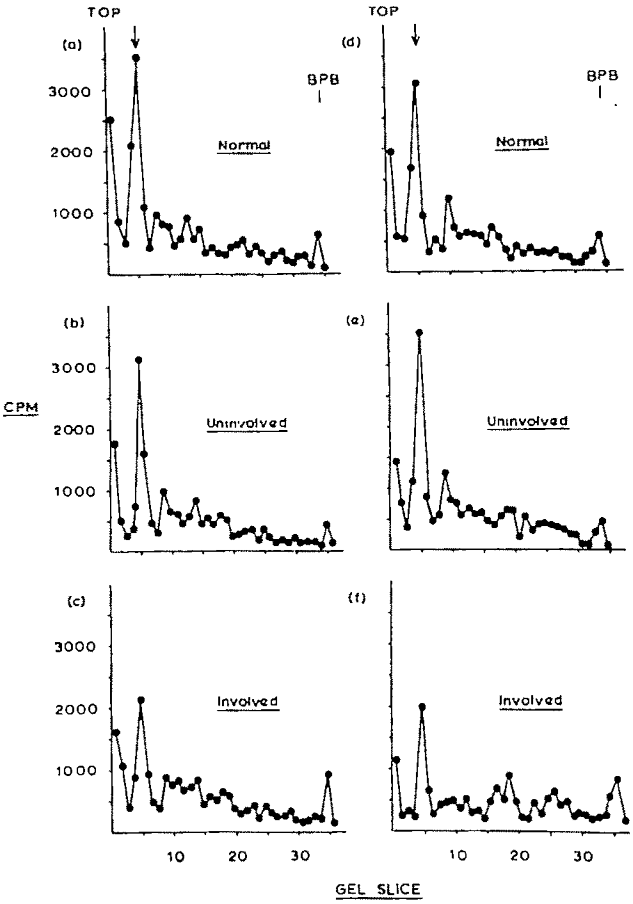

FIGURE 1. Polyacrylamide gel electrophoresis of D-(^{3}H)glucosamine labelled epidermis—two typical experiments. Samples of epidermal extracts containing 40,000 c.p.m. of D-(^{3}H)glucosamine were analysed in 7% polyacrylamide gels which were sliced into 2 mm sections and solubilized before measurement of radioactivity. The figure shows the results of two separate electrophoretic runs with different labelled samples. (a) Normal epidermis—52 year old female. (b) Uninvolved and (c) involved epidermis—40 year old male psoriatic. (d) Normal epidermis—57 year old female. (e) Uninvolved and (f) involved epidermis—49 year old female psoriatic. The arrow indicates the position of the high molecular weight peak containing proteoglycans and glycosaminoglycans. BPB indicates the position of the bromophenol blue tracking dye.

normal (Fig. 1a and d) and uninvolved (Fig. 1b and e) but was noticeably decreased in psoriatic (Fig. 1c and f) epidermis. The electrophoretic results were quantified to give values for the total labelling of this material in the whole epidermal extract (Table 3). The total incorporation of D-(^{3}H)glucosamine into this high molecular weight component in involved epidermis was significantly reduced compared to both normal and uninvolved epidermis.

The labelling of less prominent, lower molecular weight components in uninvolved epidermis was usually similar to that found in normal. However, their labelling in involved psoriatic epidermis was more variable. In about half the cases examined (e.g. Fig. 1c) the pattern of labelling was similar to normal. In the other involved samples the labelling pattern was altered with an apparent shift of

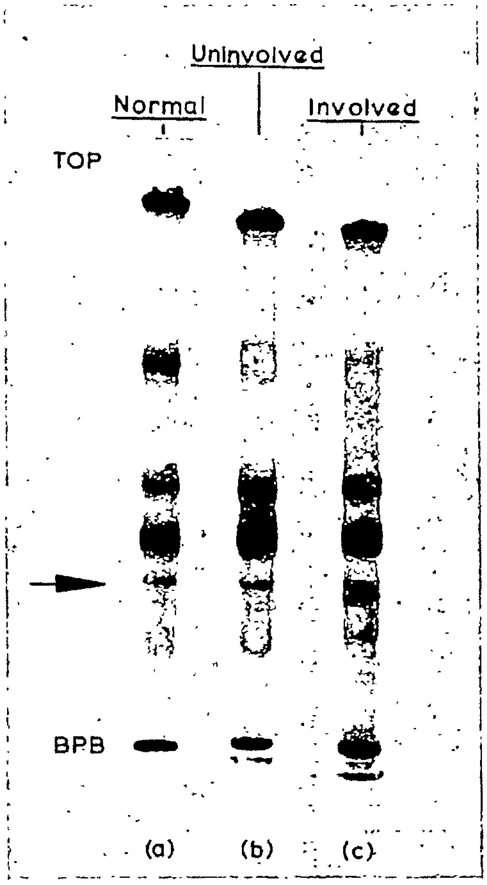

FIGURE 2. Protein composition of normal and psoriatic epidermis solubilized with 8 mol/l urea, 5% sodium dodecyl sulphate, 10 mmol 2-mercaptoethanol. Samples of epidermal extracts (50 μg protein) were run on 7% polyacrylamide gels which were stained with Coomassie brilliant blue. The arrow indicates the position of the band which occurs as a doublet in involved psoriatic epidermis. (a) Normal; (b) Uninvolved and (c) Involved psoriatic epidermis.

labelling towards the lower molecular weight end of the gel (Fig. 1f). However, no consistent changes could be detected.

Examination of gels stained with Coomassie brilliant blue revealed a consistent difference in the protein composition of involved compared to both uninvolved and normal epidermis (Fig. 2). A polypeptide molecular weight approximately 50,000 which was present as a single band in both normal (Fig. 2a) and uninvolved (Fig. 2b) epidermis always occurred as a doublet band in the involved psoriatic epidermis (Fig. 2c). This observation is similar to the findings of Thaler *et al.* (1978). Differences were also often noted in polypeptides with molecular weights less than 50,000 in the involved epidermis but these were not consistent in either their molecular weight or their abundance. In general there was an increase in the amount of polypeptides in the single band (containing components with molecular weights less than about 25,000) which migrated with the bromophenol blue tracking dye at the bottom of the gel.

DISCUSSION

We have shown previously, using pig epidermis, that the high molecular weight material heavily labelled by D-(^{3}H)glucosamine represents glycosaminoglycans and/or proteoglycan like material that is mainly extracellular (King *et al.*, 1980). Also in cultured human epidermis we have found that this material is released during trypsinization and is therefore likely to be extracellular (unpublished results). The exact relationship of this material to the cell coat that is detected histochemically is not clear. However it seems likely that this material may comprise at least a part of such a surface carbohydrate coat.

After 18 h the total incorporation of D-(^{3}H)glucosamine into the epidermis of involved psoriatic skin slices was less than into both normal and uninvolved epidermis (Tables 1 and 2). The total incorporation into the high molecular weight material was also decreased in psoriatic epidermis (Table 3). There are several alternative explanations for these observations. Firstly, the decreased incorporation may simply reflect increased dilution of the labelled sugar in what is presumably an increased pool of metabolic intermediates in psoriatic epidermis. The relative sizes of such pools are unknown at present. However this trivial explanation is unlikely since studies with other metabolic precursors (e.g. $^{32}PO_4^{2-}$, ^{14}C acetate and ^{14}C glucose) where there is also likely to be an increased pool size nearly always show an increased rate of incorporation of the precursor in psoriatic epidermis. Also studies on rat liver have indicated that a large increase in the level of sugar nucleotide intermediates may have little effect on the net incorporation of D-(^{3}H)glucosamine (Bates, Adams & Handschumacher, 1966).

Secondly, the decreased incorporation may reflect a decrease in the absolute amount and/or the rate of turnover of carbohydrates at the surface of psoriatic epidermal cells. Earlier histochemical studies have suggested a decrease in the cell-surface carbohydrates in the living layers of psoriatic epidermis and the present results would be compatible with this view. A defect in the synthesis or in the assembly of 'ground substance' carbohydrates has long been suspected in psoriasis (Cotton & Mier, 1964). It is not yet clear whether the rate of turnover of cell-surface carbohydrates is affected in psoriatic epidermis. The overall level of metabolic activity is greatly increased in psoriasis and on this basis it might be expected that the turnover of surface carbohydrates would also be increased. This point might be clarified by using double-labelling procedures to compare turnover rates directly. Under the experimental conditions used here, D-(^{3}H)glucosamine did not label cells in the horny layers of either normal or psoriatic epidermis. We are therefore unable to comment on the suggestion that cell coat material may be retained in the horny layers of psoriatic epidermis.

A third possible explanation of the decreased incorporation of D-(^{3}H)glucosamine concerns the interaction of epidermis and dermis. Using pig skin, we have shown that the synthesis of the mainly extracellular proteoglycan-like material in the epidermis requires that the dermis is present during organ culture (King & Tabiowo, 1980). When the epidermis is separated from the dermis before culture this material is not synthesized. It is possible therefore that the decreased incorporation of D-(^{3}H)-glucosamine reflects a defect in epidermal-dermal interactions in psoriatic skin.

It is not yet clear whether the altered incorporation of D-(^{3}H)glucosamine is specific for psoriasis since data on other skin diseases is not available. However, preliminary studies in this laboratory on ichthyotic skin suggest that in this condition there may be an increased incorporation of D-(^{3}H)-glucosamine into epidermal glycoconjugates. It will be necessary to examine conditions of epidermal hyperproliferation other than psoriasis to determine whether the decreased incorporation of D-(^{3}H)-glucosamine is a unique feature of psoriatic epidermis.

Most of the proteoglycans and their constituent glycosaminoglycans in skin are located in the relatively acellular dermis where they have been considered to have mainly structural functions.

However it has become increasingly clear that relatively small amounts of these materials are important components of the extracellular matrix of a variety of cell types (Kraemer, 1979) including epidermal cells (King *et al.*, 1980). These surface-associated glycosaminoglycans may play a more dynamic role in the epidermis than in the dermis. In other cell types they have been implicated in cell adhesion, migration, differentiation and organogenesis as well as in controlling cation and water binding. Thus changes to surface-associated glycosaminoglycans may have profound effects on the behaviour of cells in the epidermis. It is interesting to note that alterations in surface-associated glycosaminoglycans have also been found in tumour cells and in virally transformed cells (Saito & Uzman, 1971; Roblin *et al.*, 1975) where, as in psoriasis, cell adhesion and the control of cell proliferation are grossly abnormal.

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Sulphhydryl and disulphide stainings of subepidermal hyaline bodies

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SUMMARY

The localization of sulphhydryl (-SH) groups and disulphide (-S-S-) bonds in subepidermal hyaline bodies was examined using histochemical techniques with the specific fluorescent thiol reagent (DACM). In lichen planus, hyaline bodies showed -S-S- but not -SH staining, suggesting that cross-linking of -SH groups occurs here and favouring their derivation from epidermal cells. In lupus erythematosus and dermatomyositis, there were not only -S-S- positive hyaline bodies, considered of epidermal origin, but also -S-S- negative ones. The latter may consist of dermal components, since no dermal components are stainable with DACM. DACM staining therefore seems to be of help in determining the origin of hyaline bodies.

Subepidermal hyaline bodies (SHB), as assessed by routine histology, are eosinophilic and periodic acid-Schiff (PAS)-positive ovoid bodies seen in several inflammatory skin diseases. In order to define the nature of these structures, a number of electron microscopic and immunopathological studies have been undertaken. It has been found that they show a filamentous ultrastructure (Eady & Cowen, 1978; Ebner & Gebhart, 1972; Hashimoto, 1976) and are often associated with the deposition of immunoglobulins, complement and fibrin (Abell *et al.*, 1975; Baart de la Faille-Kuyper & Baart de la Faille, 1974; Danno *et al.*, 1980; Ueki, 1969).

In this study, the localization of sulphhydryl (-SH) groups and disulphide (-S-S-) bonds in SHB was examined, using histochemical techniques with the new fluorescent thiol reagent, N-(7-dimethylamino-4-methylcoumarinyl) maleimide (DACM) (Ogawa *et al.*, 1979). DACM has no fluorescence by itself but shows a strong and specific blue-green fluorescence (exciting wave length: 400 nm, emission wave length: 485 nm) only when it binds to -SH groups of molecules. Histochemical staining of the skin has demonstrated that -SH groups are localized mainly in the epidermal living layers whereas -S-S- bonds are shown only in the horny layer. No apparent fluorescence with DACM is detectable in the dermis and adnexae.

We therefore believe that this technique will provide further observations concerning the origin of SHB.

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MATERIALS AND METHODS

Skin specimens

Biopsy specimens were obtained from typical lesions of seven cases of lichen planus, six cases of discoid lupus erythematosus (DLE), two cases of systemic lupus erythematosus (SLE) and four cases of dermatomyositis. The tissue samples were divided, and half was immediately frozen in acetone-dry ice mixture and stored at −80°C until used. The other half was fixed in 10% formalin and processed for histological stains using haematoxylin and eosin (HE) and PAS.

DACM staining

An adjacent pair of 3μ-frozen sections were prepared. One of them was fixed in 4% formalin and stained with HE or PAS. The other unfixed section was used for -SH and -S-S- stainings according to the method of Ogawa *et al.* (1979). Briefly, for detecting tissue-bound -SH groups, a section was stained with 0·01 mM DACM solution after washing out free -SH groups. For detecting -S-S- bonds, -SH groups were first blocked by N-ethylenemaleimide. Then, the -S-S- bonds were opened using dithiothreitol and stained with DACM. HE (or PAS)-stained and DACM-stained frozen sections were compared to determine the localization of -SH groups and -S-S- bonds.

Fluorescence of -SH group-bound DACM was examined under a Nikon HFM fluorescence microscope with epi-illumination equipment, using a 200 W high pressure mercury lamp, an excitor filter (IF385–425) which excites wave lengths between 385 and 425 nm, and a barrier filter (470 K) which suppresses waves below 450 nm.

TABLE 1. DACM staining of subepidermal hyaline bodies

	Case No.	-SH staining	-S-S- staining
Lichen planus	1	−	+++
	2	−	++
	3	−	++
	4	−	+++
	5	−	+
	6	−	++
	7	−	+++
DLE	1	−	−
	2	−	−
	3	−	−
	4	−	−, +
	5	−	−, ++
	6	−	++
SLE	1	−	−
	2	−	−, +
Dermatomyositis	1	−	−, ++
	2	−	−
	3	−	−
	4	−	++

−: negative; +, ++, +++: positive fluorescence (+: slightly, ++: moderately, +++: markedly). '−, +' and '−, ++' mean that both -S-S- positive and -S-S- negative SHB coexist.

RESULTS

The results are summarized in Table 1.

Lichen planus

The localization of -SH groups was the same as that described in normal skin (Ogawa *et al.*, 1979). In no case was fluorescence for -SH groups detectable in SHB (Fig. 1).

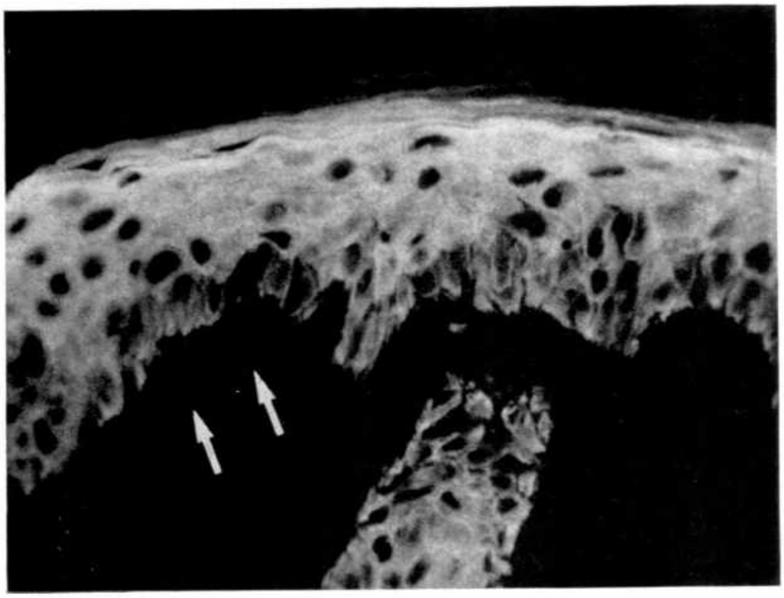

FIGURE 1. -SH staining of lichen planus. -SH groups are localized mainly in the epidermal living layers. Arrows indicate negative staining of SHB (× 800).

Fluorescence for -S-S- bonds was shown not only in the horny layer, as seen in normal skin, but also in SHB. Although the intensity of fluorescence in the horny layer and the SHB looked similar, the staining pattern was quite different: fluorescence in the horny layer took a lamellar pattern, whereas in SHB it was packed as an ovoid mass (Fig. 2). Intraepidermal hyaline bodies found in a few cases also showed -S-S- but not -SH staining (Fig. 3).

DLE, SLE and dermatomyositis

The following observations summarize the findings in these three diseases. No fluorescence for -SH groups was detectable in SHB.

With -S-S- staining, there were -S-S- positive SHB and -S-S- negative SHB: the former were present in six cases, and the latter in ten cases. Both types of SHB co-existed in two cases of DLE (cases 4 and 5), a case of SLE (case 2) and a case of dermatomyositis (case 1). Fluorescence of -S-S- positive SHB showed a packed ovoid mass.

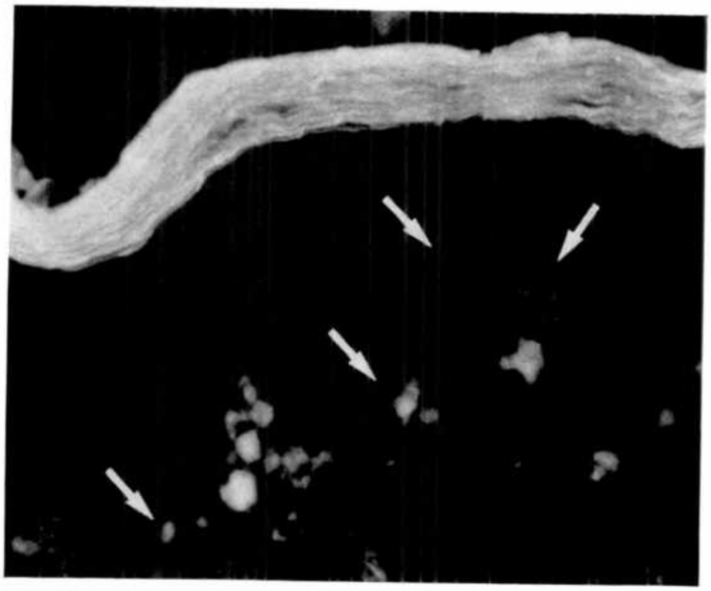

FIGURE 2. -S-S- staining of lichen planus. -S-S- bonds are localized in the horny layer and SHB. Arrows show dermo-epidermal junction (× 750).

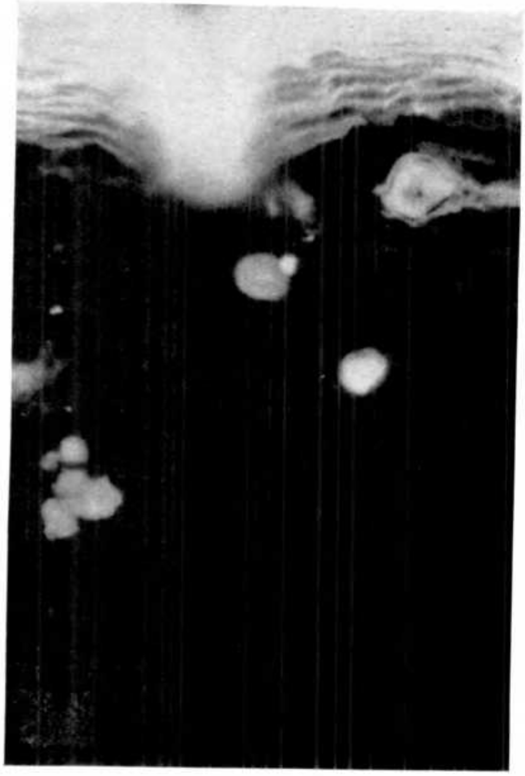

FIGURE 3. -S-S- staining of lichen planus. Hyaline bodies in the epidermis also show strong -S-S- staining (× 900).

DISCUSSION

SHB showed two different characteristics with DACM staining: -S-S- positive and -S-S- negative.

In lichen planus, Thyresson and Moberger (1957), using a modification of Barrnett–Seligman's histochemical D.D.D.-azo reaction, showed the localization of -SH components in intraepidermal hyaline bodies. Similar observations were also reported by Goltz and Hult (1963). Using more specific techniques with DACM, we demonstrated that in SHB as well as intraepidermal hyaline bodies, -SH groups make covalent bonds. Since enzymatic -SH cross-linking may take place at the stage of differentiation of keratinocytes when, in normal conditions, they are in the junctional area between living and horny layers (Ogawa *et al.*, 1979), the presence of -S-S- bonds in hyaline bodies suggests that linking of -SH groups occurs here too. The present findings thus seem to support the previous view gained mainly from electron microscopy that hyaline bodies of lichen planus are of epidermal origin (Black & Wilson Jones, 1972; Eady & Cowen, 1978; Ebner & Gebhart, 1972; El-Labban, 1970; El-Labban & Kramer, 1974; Hashimoto, 1976; Sümegi, 1979).

Fluorescence for -S-S- bonds in the horny layer was localized on the cell membrane (or intercellular spaces), whereas in the hyaline bodies fluorescence showed a diffuse ovoid mass and was not localized only on the enclosing membrane. The latter pattern of -S-S- staining was also detected in individual cell keratinization (IK) of squamous cell carcinoma (Ogawa & Taneda 1979) and sunburn cells (Danno & Horio, 1980). These structures are considered to be formed by the apoptotic process of keratinocytes: formation of membrane-bounded cell fragments followed by pyknosis of the nucleus and condensation of the cytoplasm showing aggregated tonofilaments (Hashimoto, 1976; Olson, Gaylor & Everett, 1974; Weedon, Searle & Kerr, 1979). It is therefore assumed that apoptosis may modify the pattern of -SH cross-linking in hyaline bodies, as well as in IK and sunburn cells, although no exact correlation between the histochemical changes and ultrastructural counterparts has been established.

In DLE, SLE and dermatomyositis, unlike in lichen planus in which only -S-S- positive SHB were found, there were both -S-S- positive and -S-S- negative SHB. In the former diseases, two types of SHB have been recognized: (1) SHB of epidermal origin, and (2) SHB of dermal origin including the thickened basement membrane (Lever & Schaumburg-Lever, 1975; Ueki, 1969). Our observations are in agreement with those of Ueki (1969). It is noticed here that DACM staining seems to be of value for determining the origin of SHB: (1) positive -S-S- is suggestive of epidermal origin, and (2) -S-S- negative SHB may represent those of dermal origin, as no dermal components are stainable with DACM. To validate this assumption, further studies elucidating the ultrastructure of DACM-stained and DACM-non stained SHB may be required.

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Pharmacology and Treatment

Serum levels of trimethylpsoralen after oral administration

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SUMMARY

Serum concentrations of trimethylpsoralen were determined for up to 5 h after oral administration in four patients with vitiligo. Measureable amounts of trimethylpsoralen were found in the serum only after administration of high oral doses (100 to 400 mg TMP).

For many years oral administration of trimethylpsoralen (TMP) followed by exposure to sunlight or artificial longwave ultraviolet radiation has been used for the treatment of vitiligo. The recommended dose of TMP varies between 10 and 90 mg, depending on the body weight. Very little information is available about plasma or serum levels of TMP after oral administration. Fischer, Hartvig & Bondesson (1980) developed a sensitive method based on gas chromatography followed by mass spectrometry for the determination of TMP in plasma (detection limit 2 μg/l). However, they could not detect any TMP in plasma after oral administration of 40 mg to patients. In the present study serum concentrations of TMP were determined after oral administration of the drug in various doses.

PATIENTS AND METHODS

Four patients with vitiligo were studied. None of the patients had received furocoumarins previously, and all drugs were discontinued a week before the study. Sugar coated tablets each containing 5 mg trimethylpsoralen (Trioxsalen, Elder, Ohio) were used. The TMP content of the tablets was checked by spectrophotometry (United States Pharmacopoeia). The tablets were administered in powdered form immediately after a standardized breakfast consisting of water and one jam sandwich. Blood samples were drawn at hourly intervals for up to 5 h after administration of the drug. The serum was separated and stored at −20°C until assayed. The serum concentration of TMP was measured by high performance liquid chromatography using a modification of a method which was previously

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used to measure 8-methoxypsoralen (Stolk, 1980). Extraction with heptane:dichloromethane (4:1) was carried out on 1-ml aliquots of serum after addition of diazepam as internal standard. Chromatography was performed on a reversed phase C_{18} column. The mobile phase was methanol and water (70:30), and UV detection at 254 nm was used. The detection limit of the assay was 1 μg/l.

RESULTS

Table 1 shows the serum concentrations after oral administration of different doses of TMP. After administration of 40 mg to patient 2 and 100 mg to patient 1, TMP was not detected in serum. Very low serum levels were just detectable after administration of 100 mg to patients 2 and 4 and 200 mg to patients 1, 2 and 3. Higher serum levels were achieved after administration of 300 and 400 mg TMP to patient 4. In this particular case the TMP concentrations were confirmed by repeating the high performance liquid chromatography assay, using fluorescence detection (excitation at 360 nm, emission at 430 nm) instead of u.v.-detection.

TABLE 1. Serum concentrations after oral administration of different doses of TMP

	Trimethylpsoralen serum concentration (μg/l)								
Hours after administration	Patient 1 (73 kg) T.M.P. dose:		Patient 2 (70 kg) T.M.P. dose:			Patient 3 (63 kg) T.M.P. dose:	Patient 4 (74 kg) T.M.P. dose:		
	100 mg	200 mg	40 mg	100 mg	200 mg	200 mg	100 mg	300 mg	400 mg
0	—	—	—	—	—	—	—	—	—
1	—	—	—	—	—	3	—	15	15
2	—	3	—	4	8	5	2	14	15
3	—	4	—	4	6	6	4	29	19
4	—	3	—	3	6	6	2	22	31
5	—	n.d.	n.d.	n.d.	n.d.	3	n.d.	9	10

— = Not detectable; n.d. = not determined.

DISCUSSION

We have previously shown that the serum concentration of 8-methoxypsoralen may reach a peak of 200 μg/l after administration of 40 mg of the drug by mouth (Stolk *et al.*, 1981). The data presented above indicate that the peak serum concentration of TMP achieved in humans is much lower, even when the drug is given in a high dosage. Trimethylpsoralen is not very soluble in water, and poor absorption of the drug from the gastro-intestinal tract may be responsible for the low serum levels of the drug after oral administration in therapeutic doses. This view is supported by experimental evidence in rats in which poor absorption of the drug after oral administration in a dose of 200 mg/kg body weight has been reported (Murata, Toshioka & Kita, 1970). However, it has also been found that incubation of TMP with mouse liver homogenate *in vitro* results in extensive degradation of the drug (Mandula & Pathak, 1979). The low serum concentrations of TMP may therefore also be related to rapid metabolism of the drug *in vivo*. Further investigations into the pharmacokinetics of TMP are in progress.

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Serum and saliva levels of 8-methoxypsoralen after rectal administration as a micro-enema

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SUMMARY

Serum and saliva concentrations of 8-methoxypsoralen (8-MOP) were determined in seven healthy human volunteers after rectal administration of a micro-enema. High peak serum levels were reached very soon (0·7±0·3 h) after administration of 8-MOP. Nausea was not experienced. There was a reasonably good linear correlation between 8-MOP concentrations in saliva and serum ($r = 0{\cdot}937$). The saliva/serum ratio for 8-MOP concentration was 0·08±0·02.

Psoriasis has been treated in recent years with a combination of longwave ultraviolet radiation and oral 8-methoxypsoralen (8-MOP). Usually 8-MOP is administered orally as tablets or capsules. Wide variations of 8-MOP plasma and serum levels in patients treated with 8-MOP tablets and capsules have been reported (Thune & Volden, 1977; Steiner *et al.*, 1978). Recently we (Stolk *et al.*, 1980) compared two different methods of oral administration: an 8-MOP solution and solid 8-MOP in capsules. Peak serum levels of 8-MOP were higher, reached earlier and more predictable after administration of the solution. However, nausea and abdominal discomfort are sometimes experienced after oral administration. Therefore we developed a new rectal dosage form as an alternative to oral administration. No data are available about rectal administration of 8-MOP. In a preliminary study we tested 8-MOP suppositories, using a hydrophilic and lipophilic suppository base. With both suppository bases only low 8-MOP serum levels were observed. However good results were obtained with a micro-enema. In the present study 8-MOP concentrations in serum and saliva have been determined after rectal administration of an 8-MOP solution to seven healthy human volunteers.

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MATERIALS AND METHODS

Formulation.

8-Methoxypsoralen solution:

8-Methoxypsoralen*	375 mg
Glycofurol†	25 ml
Absolute ethanol	5 ml
Distilled water	20 ml

Firstly 8-MOP was dissolved in glycofurol and subsequently the ethanol and the water were added. One ml of this solution contains 7·5 mg 8-MOP. The micro-enema was administered using a plastic disposable syringe to which a plastic application tube was connected. The syringe was filled with 7 ml of the 8-MOP solution. About 6·6 ml (50 mg 8-MOP) is delivered when the syringe is emptied.

EXPERIMENTAL PROCEDURE

Seven healthy human volunteers (one female and six male) with normal liver and renal function tests, ranging in age from 20 to 40 years (mean 32 years) and in body weight from 45 to 75 kg (mean 64 kg), participated in this study. No other medicines were taken 1 week prior to or during the tests. A blood and saliva sample were collected just before starting the experimental procedure. The experiments were initiated at noon just after eating a light lunch consisting of one cheese sandwich and a glass of water. Thereafter no food or liquid was taken until 4 h later. Irrespective of body weight a micro-enema containing 50 mg 8-MOP was administered by the volunteers themselves. No discomfort following the micro-enema was reported by any of the volunteers. They were asked to remain in a sitting position for 1 h after administration. Blood samples (about 10 ml) and at most times also saliva samples (about 2 ml) were taken at 0·5, 1·0, 1·5, 2·0 and 3·0 h after administration (subjects 1, 5 and 6) and at 0·25, 0·5, 1·0, 2·0, 3·0 and 4·0 h after administration (subjects 2, 3, 4 and 7). The serum and saliva samples were stored at −20°C until assayed. The amount of 8-MOP in serum and saliva was determined as described elsewhere (Stolk, 1980). The procedure is based on a single extraction with an organic solvent, chromatography on a reversed phase C_{18} column and u.v.-detection at 254 nm.

PHARMACOKINETIC PARAMETERS

The area under the serum concentration curve (AUC) was determined by the trapezoidal rule, from 0–4 h (subjects 2, 3, 4 and 7) or from 0–3 h (subjects 1, 5 and 6).

RESULTS

Side-effects

About half an hour after administration of the 8-MOP enema, subject 7 and to a lesser degree subject 5 complained of dizziness and a light feeling in the head. These symptoms disappeared after about 1 h. Nausea and abdominal discomfort were not experienced by any of the subjects.

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Pharmacokinetics

Serum concentration curves of 8-MOP after administration of the enema are shown in Fig. 1. Soon after administration high values of maximum serum levels (C_{max}) were observed. The values of C_{max}, the AUC and t_{max} (the time required to reach C_{max}) are demonstrated in Table 1. In an earlier study 5 months before (Stolk *et al.*, 1980) 8-MOP as a solution and as powder capsules had been administered to subjects 1, 2, 4 and 6. The C_{max}, t_{max} and AUC observed in that study are shown in Table 1 within brackets.

TABLE 1. Maximum serum concentration (C_{max}), time for serum peak concentration (t_{max}) and area under the curve (AUC) for 8-MOP given as a micro-enema

		C_{max} (μg/l)			t_{max} (h)			$AUC_{(0-4)}$ (μg.h.l^{-1})		
		Rectal	Oral (40 mg 8-MOP)		Rectal	Oral (40 mg 8-MOP)		Rectal	Oral (40 mg 8-MOP)	
Subject	8-MOP dose (mg/kg body weight)	(50 mg 8-MOP)	Sol.	Caps	(50 mg 8-MOP)	Sol.	Caps	(50 mg 8-MOP)	Sol.	Caps
1	0·76	565	(524)	(278)	0·5	(1)	(1·5)	943 (0–3)	(1129)	(585)
2	0·74	350	(127)	(97)	1	(0·75)	(2·5)	844	(286)	(233)
3	0·83	250			1			770		
4	0·74	290	(275)	(146)	0·25	(1·5)	(2·5)	394	(534)	(240)
5	0·86	525			1			1046 (0–3)		
6	0·67	191	(155)	(135)	0·5	(1)	(1)	351 (0–3)	(255)	(200)
7	1·11	694			0·5			2188		
Mean		409			0·7					
±		187			0·3					

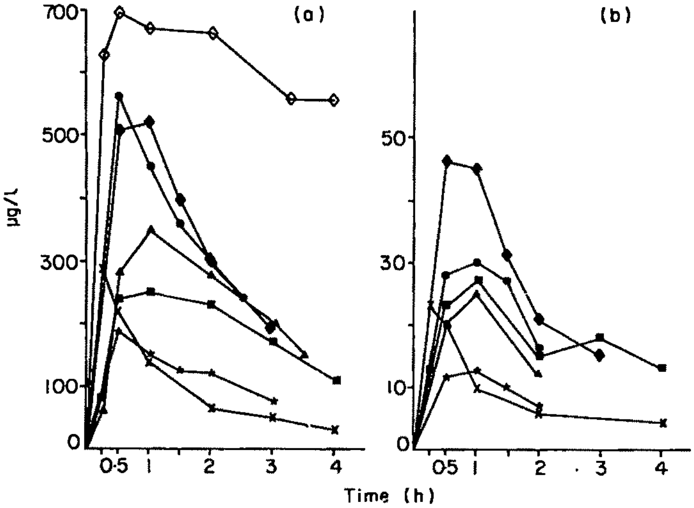

FIGURE 1. Serum (a) and saliva (b) levels of 8-MOP after rectal administration as a micro-enema. Subject 1, ●——●; subject 2, ▲——▲; subject 3, ■——■; subject 4, ×——×; subject 5, ◆——◆; subject 6, ★——★; subject 7, ◇——◇.

Figure 1 also shows saliva concentration curves for 8-MOP of all subjects except number 7. A reasonably good correlation was found between the concentrations of 8-MOP in serum and saliva of all subjects ($r = 0{\cdot}937$). The correlation of the saliva and serum concentrations of each subject and the mean ratios of the saliva and serum concentrations of each subject are summarized in Table 2. The mean ratio was 0·08±0·02 calculated by averaging all the ratios of the six subjects.

TABLE 2. Ratios and correlation coefficients of 8-MOP concentrations in saliva and serum

Subject	n	Ratio $\frac{C_{saliva}}{C_{serum}}$	Correlation coefficient
1	5	0·06±0·01	0·941
2	4	0·07±0·01	0·994
3	7	0·11±0·03	0·901
4	5	0·09±0·02	0·989
5	6	0·08±0·01	0·987
6	6	0·07±0·01	0·948
Mean		0·08±0·02	

DISCUSSION

The saliva concentration generally corresponds to the fraction of the drug in serum or plasma that is not bound to protein. The observed saliva/serum ratio of 0·08 would correspond to 8% 'free' and 92% protein-bound 8-MOP in serum. This value is in good agreement with 88–91% plasma protein binding reported by Busch *et al.* (1978). However Artuc *et al.* (1979) found 75–80% 8-MOP reversibly bound to serum proteins. The observed correlation between saliva and serum 8-MOP concentrations ($r = 0{\cdot}937$) indicates that determination of the saliva concentration may be used as an alternative to the determination of the serum concentration. Moreover the saliva concentration gives information about the 'free' biologically active fraction of 8-MOP.

A considerable individual variation of the peak serum levels and the AUC values was observed, just as we found, after oral administration of an 8-MOP solution and powder capsules (Stolk *et al.*, 1980). This large variation cannot be explained by difference in body weight of the subjects. It may be possible that 8-MOP is metabolized by the liver just after absorption: a 'first pass' effect, that varies individually. Although it is generally assumed that the liver is bypassed after rectal administration, recent investigations with other drugs indicate that the 'first pass' effect is sometimes the same after oral as after rectally administration (De Boer, 1979 & Jonkman *et al.*, 1979).

The time required to reach peak serum levels after administration of the enema was short and readily predictable (0·7±0·3 h). Peak serum levels, observed in our previous investigation of an 8-MOP solution and powder capsules, were reached respectively at 1±0·3 and 2·1±0·7 h. In conclusion, administration of 8-MOP as an enema has the advantages of rapid and predictable absorption. This makes it possible to administer 8-MOP in the hospital just before irradiation. In this way administration and the time interval between administration and irradiation can be better controlled. Gastric irritation is also avoided. However from a psychological point of view, perhaps, the unusual method of administration can be a relative disadvantage.

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Inhibition of lipase production in *Propionibacterium acnes* by sub-minimal-inhibitory concentrations of tetracycline and erythromycin

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SUMMARY

Tetracycline, declomycin and erythromycin, in concentrations lower than the minimal inhibitory dosages, were shown to inhibit lipase production by *Propionibacterium acnes in vitro.*

It is well established that the free fatty acids found in lipids extracted from human skin surfaces are formed as a result of the action of *Propionibacterium acnes* lipase on sebaceous triglycerides (Marples, Downing & Kligman, 1971). At the time, free fatty acids were considered to be the instigator of inflammation in acne vulgaris. A corollary of this thesis was the belief that the major mode of action in successful antibiotic therapy was the indirect effect of reducing free fatty acids by lowering *P. acnes* populations. Several workers also showed that *P. acnes* lipase is inhibited *in vitro* by tetracyclines, suggesting that the drop in skin surface fatty acids might be due to a direct inhibition of lipase by tetracyclines (Hassing 1971; Puhvel & Reisner, 1972; Weaber, Freedman & Eudy, 1971). The relevance of this finding was diminished, however, by the realization that the amount of tetracyclines required to inhibit lipase activity was far above therapeutically attainable doses (Puhvel & Reisner, 1972).

More recently, the significance of free fatty acids in the generation of inflammation has been questioned by the finding that amounts of free fatty acids comparable to those found in individual sebaceous follicles produce only minimal inflammation when injected intradermally (Puhvel & Sakamoto 1977a, 1977b).

Regardless of the ultimate significance of free fatty acids to acne inflammation, it is unquestionably agreed that effective antiobiotic therapy results in a reduction in levels of *P. acnes* and free fatty acids. A consistent finding, first presented by Marples *et al.* (1971), is that the reduction in the free fatty acids precedes the fall in *P. acnes* populations. One possible explanation for those findings is that in these studies bacteriostatic drugs were used and the failure to find significant reduction in *P. acnes* levels may have been due to the *in vitro* resuscitation of organisms which were inhibited *in vivo.*

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Another explanation has been suggested by the work of Mates (1973) who showed that sub-inhibitory concentrations of tetracycline inhibited the production of lipase by *Staphylococcus aureus*.

In this report, we will present data which shows that *P. acnes* lipase production is inhibited by tetracycline and erythromycin.

MATERIALS AND METHODS

Lipase assay

A modification of the lipase assay of Lawrence, Fryer & Reiter (1967) was used. A suspension of 0·02 ml Triolein (Sigma, St. Louis, MO) in 10 ml Trizma Base, pH 7·3 (Sigma)+0·05 M Ca^{++} and Mg^{++} is prepared by sonication for 30 s at 80 W in a Branson sonifier. This turbid suspension is optically stable for several hours. Equal volumes of suspension and lipase source at 37°C are mixed in a cuvette and placed in a Bausch & Lomb spectrophotometer 20 and the optical density is measured after 1 min. The decrease in turbidity in percent transmission is calculated. Lipase activity was then normalized to the weight of cells in the growth culture, expressed as log ($\triangle$% Trans/grams of cells). This erased any variation produced by subtle differences in bacterial growth.

Bacteriological methods

Propionibacterium acnes ATCC 6919 was chosen since it is well characterized and is similar to commonly found *P. acnes* (Webster & Cummins 1978). *Propionibacterium acnes* was grown in peptone-yeast-glucose broth with mineral supplements and 0·01% Tween 80 (Webster & Cummins 1978). Bacterial growth was determined turbidimetrically in a Klett-Summerson colorimeter and related to a standard curve produced by known weights of *P. acnes* cells.

The minimal inhibitory concentration for *P. acnes* 6919 of tetracycline HCl (Sigma), declomycin (Lederle) and erythromycin (Sigma) were determined by broth dilution. The highest antibiotic dosage permitting *P. acnes* growth was selected for further study. *Propionibacterium acnes* 6919 was then grown in broth in these sub-inhibitory concentrations of tetracycline (0·1 μg/ml), declomycin (0·05 μg/ml) and erythromycin (0·02 and 0·06 μg/ml). Aliquots of *P. acnes* broth culture were removed after varying time periods, the bacterial growth determined and lipase activity assayed.

RESULTS

The lipase activity in duplicate broth cultures was assayed in order to determine the reproducibility of the method. As shown in Fig. 1, lipase activity was nearly identical between the two cultures. The addition of 10 μg/ml of each of the antibiotics to culture supernatants failed to affect their lipase activity.

The effects of tetracycline HCl on lipase production are shown in Fig. 2. Production of detectable lipase activity was delayed 12 h in the presence of 0·1 μg/ml tetracycline and never reached the maximal levels of tetracycline-free *P. acnes* supernatants. The growth curves of tetracycline-treated and untreated *P. acnes* were identical.

Declomycin (0·05 μg/ml) produced a complete inhibition of detectable lipase production by *P. acnes* (Table 1) but also significantly delayed the logarithmic growth phase of the organism.

Erythromycin at 0·02 and 0·06 μg/ml produced significant depression of lipase production (Table 1). The onset of detectable lipase production was delayed by 36 h by 0·06 μg/ml and failed to reach the peak of untreated cultures. Erythromycin 0·02 μg/ml did not inhibit the onset of lipase production, but did decrease its magnitude. The growth curve of *P. acnes* was not altered by 0·02 μg/ml erythromycin, but was significantly inhibited by 0·06 μg/ml. This inhibition was manifest as a delay in the onset of logarithmic growth.

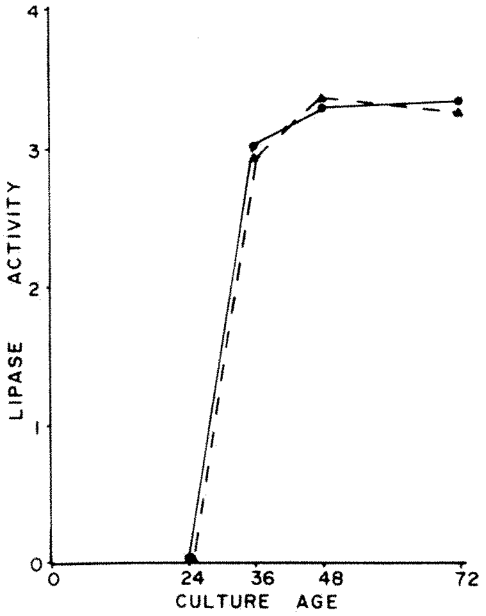

FIGURE 1. Reproducibility of lipase assay in duplicate *P. acnes* cultures.

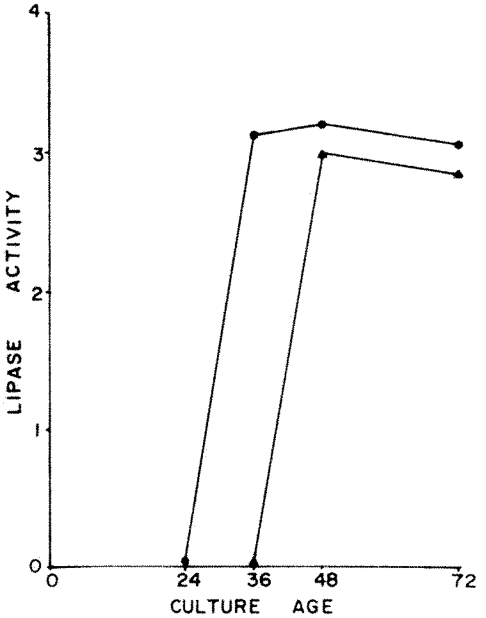

FIGURE 2. Effect of tetracycline on lipase production by *P. acnes*. ●—●, *P. acnes* grown in tetracycline-free broth; ▲—▲, *P. acnes* grown in broth containing 0·1 μg/ml tetracycline HCl.

TABLE 1. Effect of declomycin and erythromycin on *P. acnes* lipase production

	Lipase activity*				
Duration of culture†	18	36	48	60	72
No antibiotic	0	3·74	3·10	3·15	2·75
0·02 μg/ml erythromycin	0	3·28	2·95	3·15	2·71
0·06 μg/ml erythromycin	0	0	0	3·53	3·35
0·05 μg/ml declomycin	0	0	0	0	0

* Expressed as the log [(Δ% Transmission)/grams of cells]; † in hours.

DISCUSSION

We have found that 'sub-inhibitory' concentrations of tetracycline HCl, declomycin and erythromycin base all inhibit lipase production by *P. acnes*. These data confirm a phenomenon described for staphylococcal lipase (Mates 1973) and suggest a mechanism for the fall in skin surface fatty acids which precedes the drop in *P. acnes* populations during antibiotic therapy.

It is extremely unlikely that the low levels of antibiotic in the culture supernatant resulted in the direct lipase inhibition, since they were at least 60-fold lower than the minimal lipase-inhibitory concentration reported by Puhvel & Reisner (1972). Our data support this. Addition of 10 μg/ml of each of the antibiotics failed to decrease lipase activity. Moreover, the added Ca^{++} and Mg^{++} in the triolein suspension would overcome any cation chelation by tetracycline (Weaber, Freedman & Eudy, 1971). Thus, decreased lipase levels in antibiotic-treated cultures are not the artifactual result of residual antibiotic on the lipase assay.

Our expression of lipase activity normalized to the *P. acnes* population is designed to overcome subtle perturbations in growth due to the presence of antibiotics. However, the growth curve depression caused by 0·05 μg/ml declomycin and 0·06 μg/ml erythromycin could be too pronounced to permit valid comparison to untreated *P. acnes* cultures. The magnitude of lipase inhibition produced by these treatments could thus reflect growth suppression and not only inhibition of production. Whichever is the case, the data indicate that *P. acnes* grown in the presence of 'sub-inhibitory' amounts of antibiotic have decreased lipase production.

Current thought holds that fatty acids produced by *P. acnes* have, at most, a minor role in the inflammatory stage of acne (Puhvel & Sakamoto, 1977a, 1977b). Production of neutrophil chemotactic factors (Puhvel & Sakamoto, 1978), activation of complement (Massey, Mowbray & Noble, 1978; Webster *et al.*, 1978, 1979a) and triggering of lysosomal hydrolase release (Webster, Tsai & Leyden, 1979b) have recently been implicated as potential phlogistic roles for *P. acnes* in acne. It is possible that protein synthesis-inhibiting antibiotics, such as tetracycline and erythromycin, could decrease the production of microbial inflammatory factors and thus reduce *P. acnes* inflammatory capacity before a change in bacterial numbers is evident.

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Case Reports

Zinc deficiency with transitory acrodermatitis enteropathica in a boy of low birth weight

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SUMMARY

A premature male baby fed on his mother's milk developed zinc deficiency and a skin disorder inseparable from acrodermatitis enteropathica. Following zinc therapy the skin lesions healed. Later the treatment was withheld and no recurrence was seen during 30 months' observation. The boy's zinc deficiency was thought to be due to a high requirement secondary to rapid growth, to poor zinc supply in food and, possibly, to inefficient zinc absorbtion.

Acrodermatitis enteropathica (AE) is a rare hereditary chronic disorder which starts in early infancy after weaning and is characterized by alopecia and skin lesions localized to periorificial areas and acrally on the extremities. Diarrhoea is often present, growth retarded and the general state of health is poor. Recent reports show that AE is a zinc deficiency disorder which can be cured by daily oral zinc supplements (Barnes & Moynahan, 1973; Michaelsson, 1974; Thyresson, 1974; Neldner & Hambidge, 1975). Lombeck *et al.* (1975) found impaired intestinal absorption of ^{65}Zn but no increase of zinc losses in patients with AE.

Transitory AE-like disorders secondary to other diseases have been described. The two patients reported by Weismann, Fischer & Hjorth (1976) were maintained on total parenteral nutrition because of severe Crohn's disease. No zinc supplementation was given initially and AE-like lesions developed after 6–8 weeks. Following zinc supplementation the skin healed rapidly. Sivasubramanian *et al.* (1978) described similar lesions in a premature infant who was maintained on parenteral nutrition for 5 weeks.

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CASE REPORT

History

SS, a boy, was born after 30 weeks gestation. His birthweight was 1220 g. Since the mother was included in a study of Jameson *et al.* (1977) the concentration of zinc had been measured in her serum at 14 weeks gestation and in the boy's cord blood. The boy gained weight as expected up to the age of 15 weeks when his normal weight gain slowed down (Table 1). He was still being fed breast milk when the symptoms of the disease started around one of his ears and perianally. The dermatitis spread rapidly and the child was referred to hospital at the age of 20 weeks. By this time he had well demarcated, erythematous, scaly and partly crusty skin lesions around the mouth, nose, ears, anus and genitalia, at the lateral corners of his eyes and on the extensor surfaces of his fingers and toes and even on his knees and elbows. Scalp hair, eyebrows and eyelashes were normal. It was also observed that the child did not fix gaze. His stools were normal. Since the clinical picture was similar to AE, zinc analyses were made. The serum zinc concentration was only 5 μmol/l and oral supplementation was immediately started. 15 mg zinc was given daily as zinc sulphate, divided into three doses (Solvezink, AB Tika, Lund, Sweden). Within 24 h there was an obvious improvement of the skin

TABLE 1. Patient's weight gain. Zinc was given from the 21st week after delivery

Age (weeks)	Mean gain g per week
2–6	177
6–9	110
9–14	120
14–20	90
22–25	166
25–28	158
28–37	107

TABLE 2. Some data on the patient

Age (weeks)	Newborn	20	22	23	25	28	37	46	55	79
Dermatitis	0	+++	(+)	0	0	0	0	0	0	0
Treatment mg Zn^{2+} daily	0	0	15	7.5	5	5	5	5	0	0
Zinc in serum μmol/l	20	5	30	20	28	33	26	19	19	19
Copper in serum μmol/l	13	13	–	13	–	–	–	–	–	21
Alkaline phosphatase in serum μkat/l	–	6	28	–	51	–	–	–	–	13.5
Alkaline phosphatase in leukocytes arbitrary units	–	8	–	100	25	45	–	–	–	15

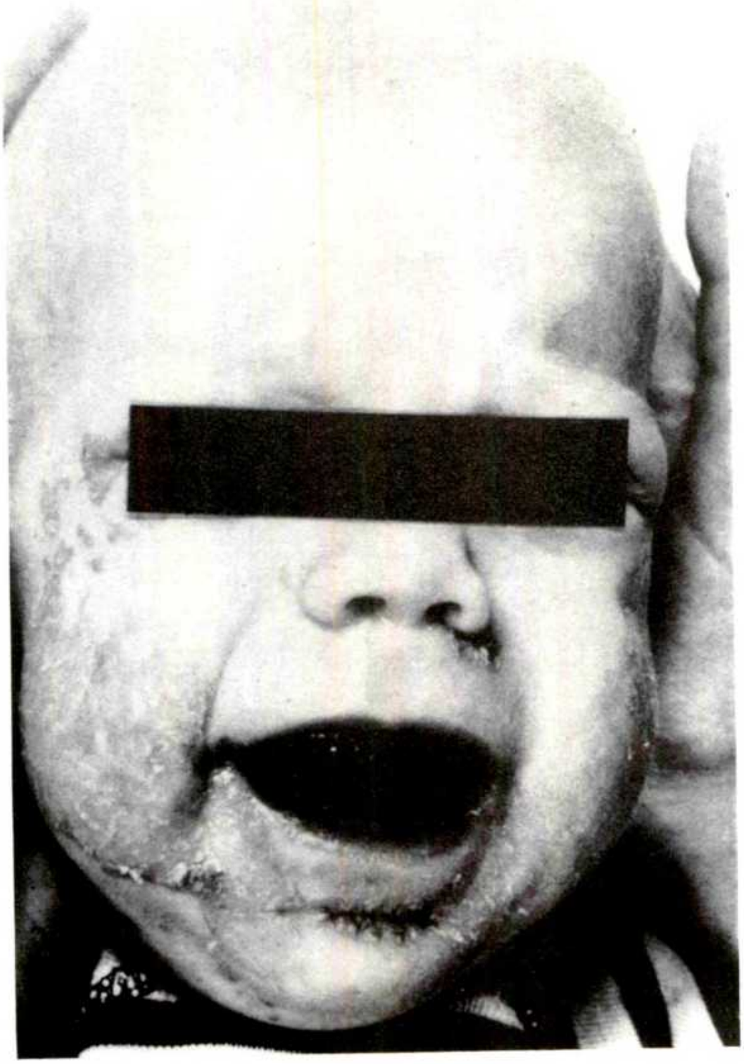

FIGURE 1. Patient before start of zinc treatment.

lesions and after 4 days of treatment only a slight erythematous shade marked the site of earlier changes. The clinical course is illustrated by Figs 1–2. After 7 days' treatment the daily dose of zinc was reduced to 10 mg and after an additional 9 days to 5 mg. At the age of 1 year all zinc supplementation could be withheld without recurrence of his skin disease. Serum zinc concentrations and alkaline phosphatase activities during and after treatment are shown in Table 2. Initial serum alkaline phosphatase activity was considerably low and rose dramatically to very high levels when oral zinc was given. Alkaline phosphatase activity in neutrophil granulocytes, using the method described by Kaplow (1955), showed a low score and rose considerably after zinc treatment. Routine laboratory tests including serum albumin were normal.

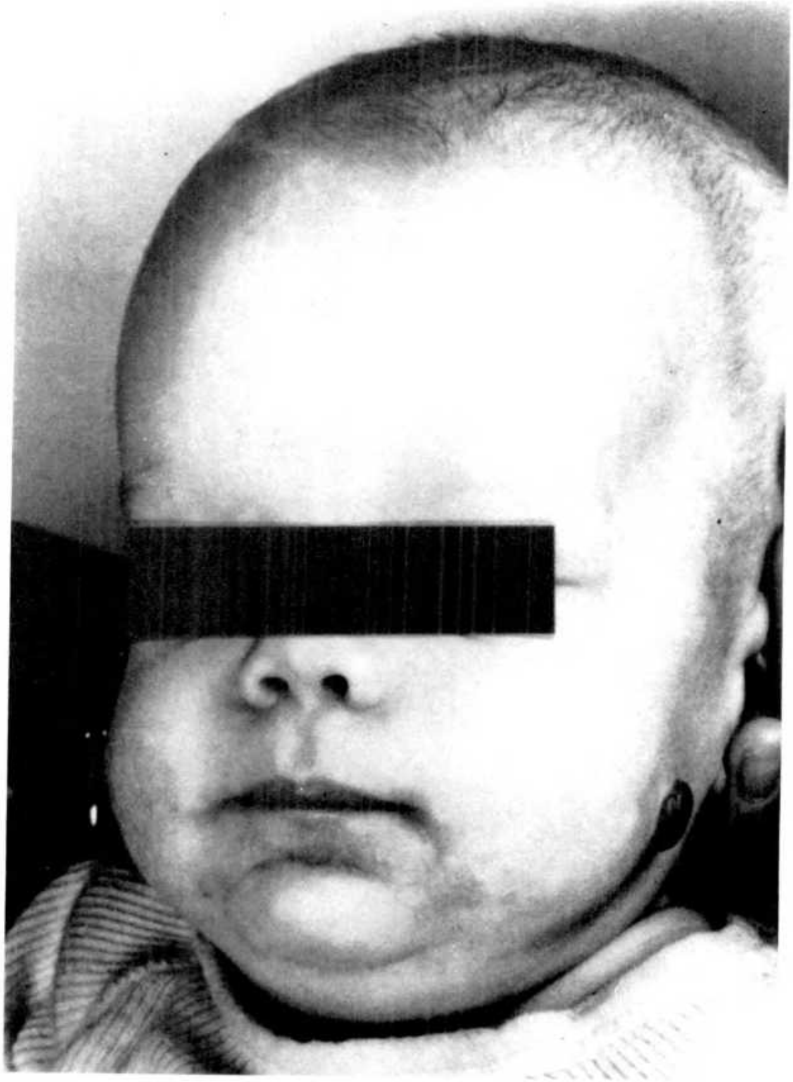

FIGURE 2. Patient after 5 days of zinc treatment.

Feedings

Up to 40 weeks of age the boy was fed mainly his mother's milk. At admission, two samples of milk contained 4 and 6 μmol zinc/l respectively. A whey-protein formula (Milkotal) was also given although in small quantities. This contained 15 μmol zinc/l. During the next 10 weeks increasing quantities of cow's milk formula containing cereal were given but breast feeding was not discontinued until the age of 11 months. Then the boy had increasing quantities of the family's ordinary food.

Family history

The patient had one older, healthy brother who was also breast fed but whose birthweight was normal. The parents were healthy and free from skin or bowel disorders.

DISCUSSION

The diagnosis of AE was made by the clinical and biochemical findings and by the rapid healing that followed zinc treatment. In some details, however, this boy differs from AE as usually described. In classical AE the disease starts after weaning and is life-long whereas our patient was still being fed breast milk when symptoms started and treatment could be stopped without relapse of the disease. It seems improbable that the boy as a newborn or his mother could have been zinc deficient. The mother's serum zinc was 10·4 μmol/l at 14 weeks of pregnancy and 14 μmol after delivery. The boy's cord blood contained 20 μmol/l, a high normal value compared to the 14·6±3·4 found in 105 newborns studied by S.Jameson (unpublished data). Henkin, Marshall and Meret (1971) studying 15 newborns found 13±4 μmol/l. Most likely the boy developed zinc deficiency postnatally.

The zinc concentrations of the mother's milk were 4 and 6 μmoles on two occasions, which are low values when compared to standard reference books (Bergmann & Fomon, 1974). However, studies by Ekman & Palm (1974) and by Vuori & Kuitunen (1979) have shown that zinc concentration decreases during lactation. The latter authors found 4–30 μmol/l after 20–22 weeks. Thus our patient was fed milk that had a low but still normal zinc content.

Dauncey, Shaw & Urman (1977) have studied zinc balance in infants of very low birth weight. In those born prematurely, negative balance was found from the second to ninth week, while in those born near term, a much earlier change to positive balance was found. The authors concluded that deficient zinc absorption is a frequent finding in the very premature, rapidly growing baby and predicted that clinical disease could be expected to be found in the future. Our patient seems to confirm their prediction.

The care of the very premature newborn baby has been much improved during recent years and survival is now common. Babies are usually fed human milk, if possible their own mothers'. Dermatologists and paediatricians must be aware of the possibility of zinc deficiency in these babies.

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Acne induced by dantrolene

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SUMMARY

We describe two cases of an unusual acneiform eruption which occurred in middle aged women being treated with dantrolene for spasticity.

Acneiform eruptions are seen as a side-effect of many drugs, including bromides and iodides, steroids, and isoniazid. We have recently seen two patients, both middle-aged women, who developed an unusual acneiform eruption, mainly of darkly pigmented blackheads, during treatment with dantrolene (Dantrium) for spasticity.

Case 1[*]

A 39-year old laboratory technician of mixed English and West Indian descent, with no previous history of acne, was first seen in June 1978 complaining of blackheads on the face and painful 'boils' on the buttocks. In January 1975, she had taken an overdose of tricyclic antidepressants, and on recovery was found to have a spastic paraplegia with a sensory level at D8. Her rehabilitation was complicated by severe spasticity and painful flexor spasms, which responded poorly to diazepam and baclofen. Treatment with dantrolene was started in April 1975, and gave great relief; the dose was gradually increased to 425 mg daily. Blackheads first appeared on the face in the autumn of 1975, and in the summer of 1977 occasional painful swellings began to appear on the buttocks and the perineum. Some of these were incised while others burst spontaneously, discharging pus for 2 weeks and then resolving; no pathogenic bacteria were cultured from the pus on any occasion.

Examination revealed an eruption, principally of blackheads, with a few closed comedones, inflammatory papules, and an occasional pustule over the cheeks (Fig. 1), the buttocks and perineum, and that part of the dorsal spine which rested against her wheelchair.

Investigation. Full blood count, ESR, urea and electrolytes, liver function tests, plasma testosterone, serum bromides and iodides were all normal. Histology of the skin was unremarkable apart from the presence of keratin-filled cysts communicating with the surface.

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[*] Shown to the Dermatological Section of the Royal Society of Medicine, October 1978.

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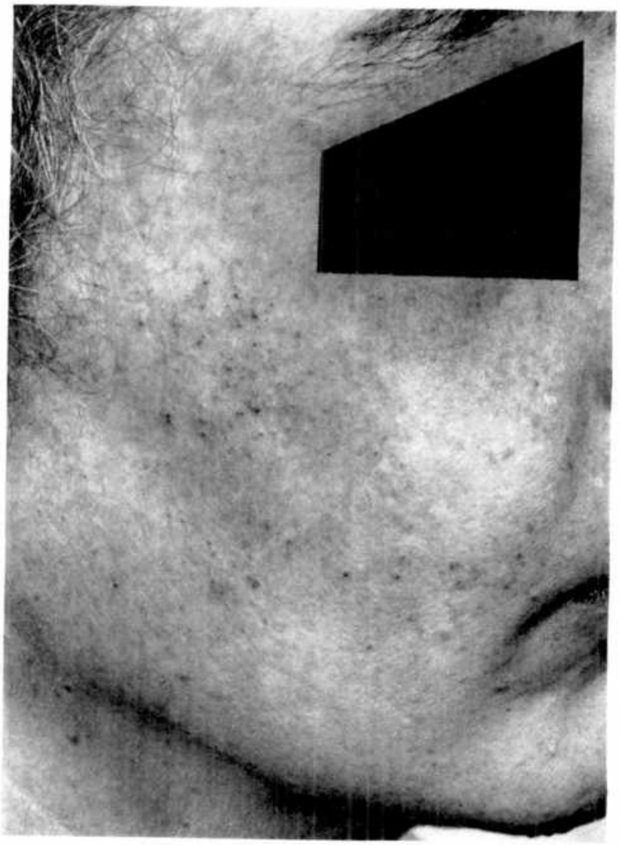

FIGURE 1. (Case 1) Open and closed comedones over cheeks.

Treatment and progress. Over the last 18 months she has continued the same dose of dantrolene. On several occasions she has had one or two tender cystic swellings over the buttocks and perineum. Pus can be aspirated from these and has always been sterile on culture. Their resolution has been hastened by local injection of triamcinolone acetonide. The incidence of these swellings has been reduced by the addition of oxytetracycline 250 mg twice daily to her other treatment. The blackheads have diminished on her face, but have increased considerably over sites of pressure, such as the extensor aspects of her forearms and the backs of her thighs, and at sites of friction by clothing, especially under her brassiere and shoulder-straps (Fig. 2).

Case 2

A 51-year old Caucasian woman was referred in December 1975 having noticed a dark area on her right cheek. She had suffered from disseminated sclerosis since 1953, and had been confined to bed for most of the time; because of a painful scar from a bed-sore, she invariably lay on her right side. She gave no previous history of acne but had had occasional episodes of seborrhoeic dermatitis of the face. Dantrolene was started in January 1975 for flexor spasms of the legs, and increased to a dose of 150 mg daily.

Examination revealed a fairly well defined area of pigmentation and scaling with very numerous blackheads and a few giant comedones. The rest of the face and her body were normal.

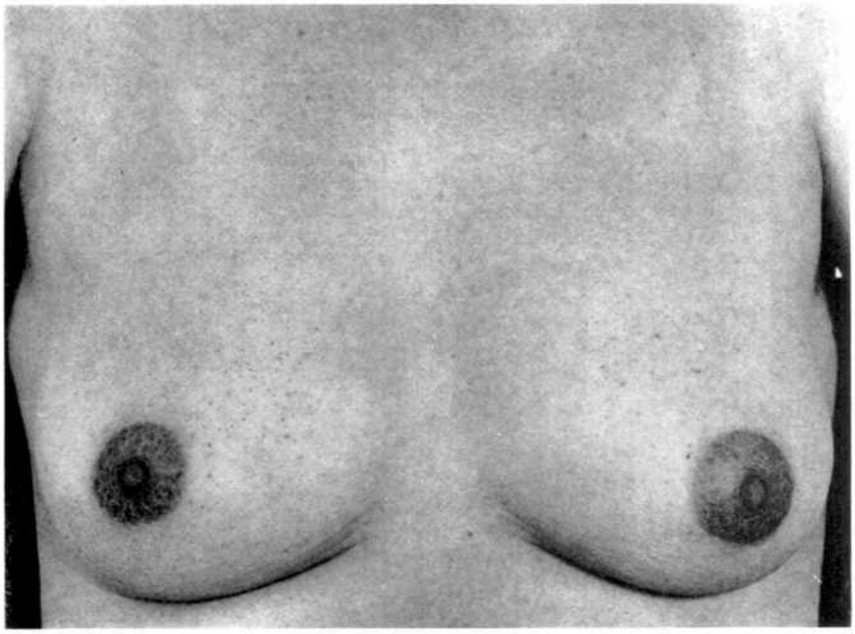

FIGURE 2. (Case 1) Prominent blackheads over the breasts, under the brassiere.

DISCUSSION

The acne in both these cases favoured sites of chronic trauma and friction, but differed in its clinical pattern from the usual acne mechanica which is commoner in younger, acne-prone subjects. No comedogenic agents were being applied to the skin, and the late onset, severity and unusual pattern of acne led us to suspect a drug in both cases. Joynt (1976) described acneiform eruptions in nine out of seventy-seven patients receiving dantrolene, and noted the predilection for areas subject to pressure such as the back and the extensor aspect of the forearms. It is likely that our two patients are also suffering from acne induced by dantrolene as in both cases it started 6 months after starting the drug, but it has not been justifiable to try stopping treatment for some months.

We also considered the possibility that the acne might be due not to the drug itself but to iodine derived from the capsule shells, which are coloured with erythrosine, the monohydrate of the disodium salt of 2, 4, 5, 7-tetraiodofluorescein. Capsules containing this dye may cause an elevation of the serum protein-bound iodine, which led to confusion when this was the standard test of thyroid function (Haas, 1970; Bora, Rabichevich & Werner, 1969). However, erythrosine is a dye which has been permitted for use in food and drugs for many years. Confidential information from other manufacturers reveals that larger quantities are present in formulations of many common drugs, some of which may be taken continuously for years, but have not been reported to cause acneiform eruptions. Nevertheless, Case 2 has noticed subjective improvement since taking the filling of the capsules without the shells, but this could have been because she has been encouraged at the same time to spend longer sitting out of bed.

It therefore is most probable that the acne is caused by the drug dantrolene itself. There is no evidence of virilization in either case, or of liver disease (although this has been reported in some patients taking dantrolene) and the mechanism remains obscure. It is interesting to note that dantrolene is a hydantoin, and so is chemically related to phenytoin, which may also be acneigenic (Jenkins & Ratner, 1972; Frentz, 1977; Walshe, 1972).

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Richner-Hanhart syndrome (tyrosinaemia-II) (report of four cases without ocular involvement)

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SUMMARY

Four cases of tyrosinaemia with cutaneous manifestations, but without ocular involvement, are reported in a family with consanguineous parents. The tyrosine levels in the serum and in the urine were normal in both parents, while in the offsprings the tyrosine levels were elevated 7½–13 times in the serum and 3–13 times in the urine. Although the serum tyrosine levels in our cases were higher than in most of the cases reported in the literature the eyes of all our patients were normal. The skin manifestations were very impressive, and varying degrees of mental retardation were present in all patients. The patients put on a low-protein diet improved considerably and have been kept symptom-free for 1½ years. The possible implication of the discrepancy between the high serum levels and lack of ocular changes is discussed. Our results suggest that the Richner-Hanhart syndrome may include more than one distinct biochemical entity.

INTRODUCTION

Tyrosinaemia type II is an inherited autosomal recessive disorder known as Richner-Hanhart syndrome, characterized by persistant tyrosinaemia, tyrosinuria and normal liver and kidney function. The blood tyrosine level may reach 25–30 mg/100 ml compared to normal levels of 2·5 mg/100 ml (Zaleski, Hill & Kushniruk, 1973) while the urinary excretion may reach as high as 410 mg/24 h (Goldsmith *et al.* 1973). A presumptive clinical diagnosis may be confirmed by a simple screening test with nitrosonaphthol (Scriver & Rosenberg, 1973). In this syndrome consanguinity has been reported frequently. The syndrome is characterized clinically by pseudodendritic corneal opacities, painful punctate palmo-plantar keratosis and mental retardation (Goldsmith *et al.* 1973). The eye lesions may occur in infants as early as the age of 2–4 weeks (Goldsmith & Reed, 1976). The eyes become painful and photophobic and show lacrimation, nystagmus, and white thickened conjunctivae with discrete conjunctival plaques. The cornea becomes hazy or shows severe keratitis and pseudoherpetic dendritic ulcerations with negative fluorescein staining and negative culture for viruses. The lens may show subcapsular dots or opacities.

The skin lesions may occur as early as 6 weeks or as late as 6 years. They are usually confined to

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palms and soles and are painful. They appear as discrete hyperkeratotic papules with an irregular polycyclic border and are located mainly on peripheral pressure bearing points. In other instances they may even begin as painful erosions which become crusted and hyperkeratotic. Hyperkeratotic papules of linear pattern have been described on the flexor aspect of fingers or toes, surrounded by erythema and desquamative hyperkeratosis, and fissures were found over joints. The palms can be hyperhidrotic and uniformly hyperkeratotic. Hyperkeratotic slightly raised plaques in the prepatellar region and over the elbows have also been reported. The hair and nails are normal.

The syndrome is also often associated with permanent neurological damage, mental retardation and other anomalies such as microcephaly, cleft lip, cleft palate, inguinal hernia, absence of one kidney and talipes equinovarus (Kennaway & Buist, 1971).

CASE HISTORY

Four siblings of a family were investigated with the main complaint of palmar and plantar keratosis showing fluctuation in severity. They were F.Kh. 22 years, female; A.K.Kh. 19 years, male; A.Kh. 15 years, male and H.Kh. 10 years, female.

The skin manifestations were uniform in all of the patients. The lesions were confined mainly to the soles and palms and the patients noticed the pattern of the lesions changing over a period of time. There were ill-defined hyperkeratoses and lamellar peeling of the skin combined with fissuring.

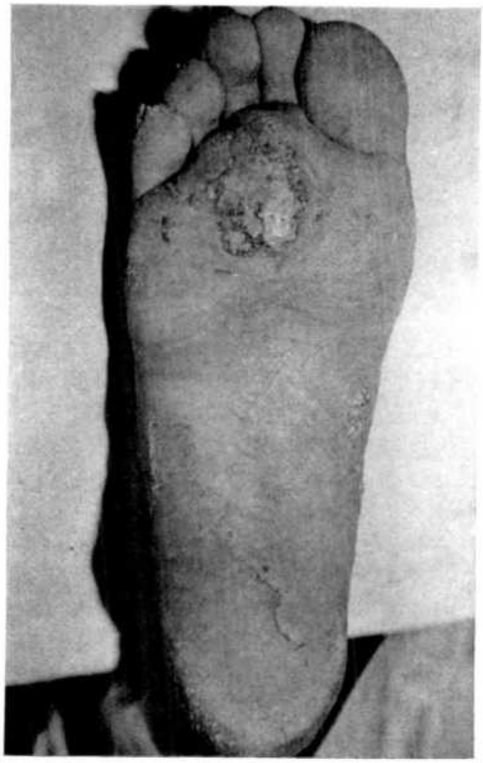

FIGURE 1. Patient A.K.Kh., 18 years, showing the severest involvement as compared with other siblings (before diet).

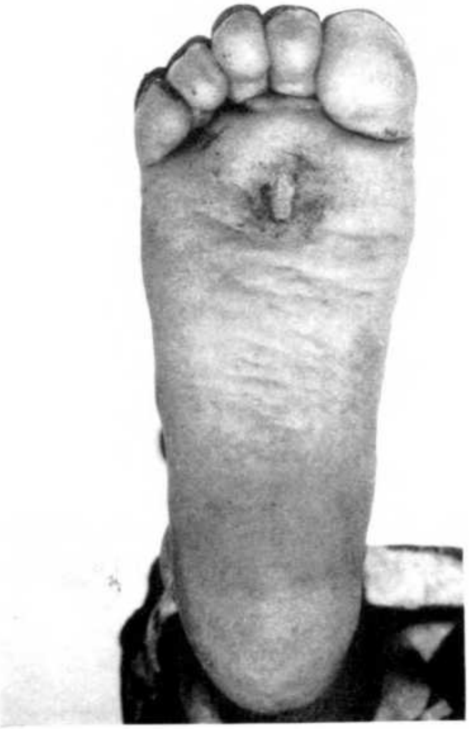

FIGURE 2. The same patient A.K.Kh., 18 years, after 6 months on the diet.

In some of the patients a peculiar yellow-green discoloration of the involved sites was conspicuous. The lesions were tender and sometimes painful enough to prevent the patients standing and make them crawl on their knees (Figs 1, 2). The changes started in all of the patients at 1½ years of age except the youngest girl H.Kh. aged 10 years whose first complaint began at the age of 4 years. Physical examination did not reveal additional abnormalities such as brachytelephalangia, lipomata or subungual keratoses. Moderate hyperhidrosis was found in A.Kh. the 15 year old boy. All the offspring were pale and had fair hair as babies. The parents were first cousins (Fig. 3).

METHODS

Ophthalmological examination (including the slit lamp), psychological tests, routine laboratory investigations (RBC, WBC, differential count, platelets, urine, liver function tests, serum electrolytes, blood sugar, urea), estimation of tyrosine levels in serum and in 24 h urine were performed in all the patients, their parents and unaffected brother. The screening nitrosonaphthol test (2·63 N nitric acid 1 ml + 1 drop 2·5% sodium nitrate + 10 drops of nitrosonaphthol reagent + 3 drops of urine) was performed. The qualitative tests were performed by thin layer chromatography on cellulose acetate plates with N-butanol/acetone/acetic acid/water as a solvent and ninhydrin as a locating reagent.

Quantitative amino acid analysis was done in 24 h samples of frozen urine and in blood samples taken in plain tubes after overnight fasting, then the separated serum was deproteinized with sulpho-

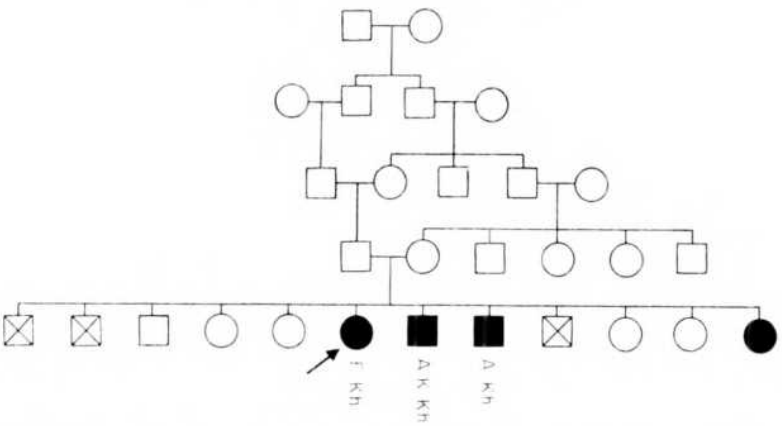

FIGURE 3. Kh. family pedigree. Following the family pedigree to the fourth offspring shows:
1. Parents of patients are first cousins.
2. Grandfather of siblings (mother's side) is the brother of grandmother (father's side).
3. Great grandfathers of offspring are brothers.
☒, aborted male; ■, affected male; ●, affected female.

salicylic acid and stored at −20°C. Analysis was performed by Beckman single column automatic amino acid analyser 119 CL.

RESULTS

The screening nitrosonaphthol test was positive in the urine of each of the four patients, but negative in the parents.

Qualitative amino acid analysis performed by thin layer chromatography using cellulose acetate plates showed increased tyrosine in the serum and the urine of all the patients (Fig. 4).

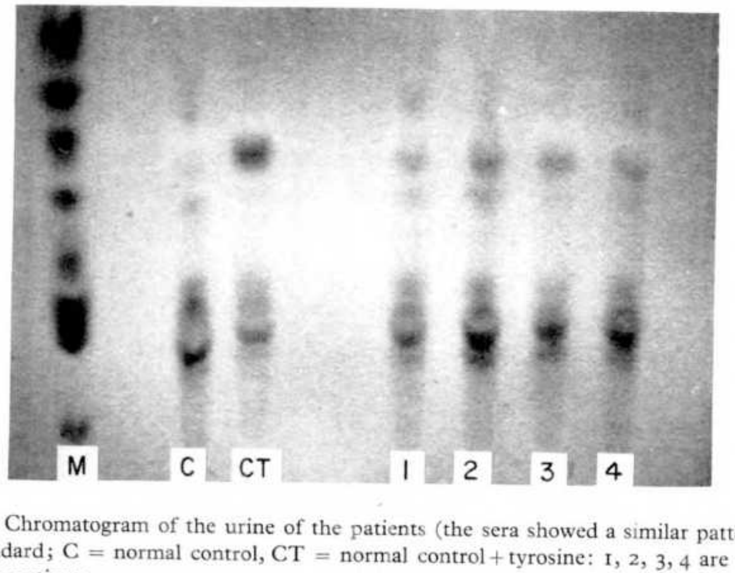

FIGURE 4. Chromatogram of the urine of the patients (the sera showed a similar pattern). M = Mixed standard; C = normal control, CT = normal control + tyrosine: 1, 2, 3, 4 are samples of urine of the patients.

Quantitative tyrosine assays showed that the serum tyrosine was elevated 7·5–12 times in patients as compared to normals. The 24-h urine tyrosine level was elevated 3–13 times as compared to normal (see Table 1).

TABLE 1. 24 h urine tyrosine levels

Subject		Tyrosine level (mg%)	
		Serum	24 h urine
Normal adult		0·8–1·3	12–28
F.Kh.	22 years	7·4	169
A.K.Kh.	19 years	11·4	600
A.Kh.	15 years	8·6	553
H.Kh.	10 years	6·7	111
Mean		8·5	358
Mother		1·1	46
Father		0·52	36
Brother	33 years	1·06	–

Other laboratory investigations were all within normal limits. Ophthalmological examination did not reveal any abnormalities. Psychological tests showed retarded development with IQ in marginal ranges.

The findings confirmed tyrosinaemia with the clinical characteristics of the Richner-Hanhart syndrome except for the lack of ophthalmological involvement.

FOLLOW-UP

The patients were put on a restricted protein diet (low tyrosine content) supplied by the department of dietetics.

Regular follow ups were done during a period of 1½ years. The skin condition of all the patients considerably improved, the hyperkeratoses were no longer uncomfortable and the patients were able to walk. Serum and urine screening after 12 months of the dietary regimen showed normal tyrosine levels.

DISCUSSION

From the differential diagnostic point of view other clinical types of plantar and palmar keratosis were considered. Hyperkeratosis palmoplantaris is a disorder which is sometimes of autosomal recessive nature as in mal de Meleda and Papillon-Lefèvre syndrome. If the disorder is of late onset the possibility of association with oesophageal cancer should be born in mind (Harper, 1971). Should the palmoplantar hyperkeratosis be painful the diagnosis of Richner-Hanhart syndrome has to be considered. Tyrosine increases in the blood in tyrosinaemia I (tyrosinosis) which is a rare metabolic disorder of autosomal recessive inheritance and is associated with splenomegaly, hepatomegaly, cirrhosis, fever, oedema, vomiting, renal glycosuria, gross generalized amino aciduria, phosphaturia and renal rickets. The levels of tyrosine and sometimes methionine are increased in the blood, liver function tests are abnormal and the patients do not show any of the characteristic skin or eye manifestations.

Neonatal tyrosinaemia is a disease that is usually seen in premature infants. It is usually transient and is associated with lethargy but without skin or eye lesions. It responds dramatically to ascorbic acid treatment. The symptoms and laboratory investigations recorded in our patients are thus consistent with type II tyrosinaemia (Richner-Hanhart syndrome), although the nature of the metabolic error giving rise to this particular combination of signs without ocular involvement is still open to question. The current concept of tyrosine metabolism involves major degradation by tyrosine amino transferase to parahydroxyphenylpyruvic acid, homogentisic acid and acetoacetic acid and synthesis along other pathways to melanin, noradrenaline and adrenaline and to thyroxine. The enzymatic basis of tyrosinaemia associated with corneal ulcers in one case reported by Burns (1972) was due to a deficiency in enzyme soluble tyrosine aminotransferase. In order to find out the nature of the genetic metabolic error further biochemical tests have to be carried out, such as assays for parahydroxyphenylpyruvic acid, parahydroxyphenyllacetic acid and parahydroxyphenylacetic acid. It is also desirable to complete the investigation by enzyme studies on tissue biopsies for parahydroxyphenylpyruvic acid oxidase and tyrosine aminotransferase.

The difference in the clinical picture between the various types of hepatic tyrosinaemia type I could be tentatively explained by the existence of several isozymes under individual genetic control as suggested by Kennaway & Buist (1971).

The incomplete manifestations in our cases which showed complete lack of ocular involvement combined with strikingly high serum levels of tyrosine support the possibility of divergent involvement of enzymes in metabolic errors not only in the group of hepatic tyrosinaemia (type I) but also in tyrosinaemia type II (Richner-Hanhart syndrome).

The approach to the treatment of genetic disorders in dermatology is rather depressing. The genotypic expression (phenotype) of some disorders could be corrected by dietary measures as in phenylketonuria, acrodermatitis enteropathica, Refsum's syndrome and the Richner-Hanhart syndrome. Early and correct diagnosis in such cases is mandatory and by treatment major complications such as severe mental retardation could be avoided. As our knowledge and understanding of the basic biochemical defects increase further therapeutic measures will be available and the outlook will be better.

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Demethylchlortetracycline and griseofulvin as examples of specific treatment for mycosis fungoides

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SUMMARY

Long-term control of mycosis fungoides in the tumour stage was achieved in one patient by daily demethylchlortetracycline therapy. On two occasions widespread nodules and tumours completely involuted within 1 month of initiating this therapy. A second patient showed complete resolution of his plaque stage mycosis fungoides after the institution of oral griseofulvin therapy for a chronic fungus infection. The disease reappeared upon stopping the griseofulvin and involuted upon its resumption.

The observations are interpreted as evidence that reduction or eradication of a persistent bacterial or fungal antigen may have a remarkable ameliorative effect in selected mycosis fungoides patients.

The knowledge that mycosis fungoides is a malignant growth of lymphoid tissue has sharply defined the reach of the therapist. He feels the need to restrict his therapy to lymphocytoxic measures such as topical nitrogen mustard, radiotherapy, or chemotherapy (Winkelmann & Caro, 1977). The present report discloses an entirely different therapeutic approach which supports the concept that mycosis fungoides may result from the presence of a persistent antigen in a sensitized patient. In the two patients to be reported, long-term specific antibiotic therapy proved remarkably effective in controlling the disease.

CASE REPORTS

Case 1

In 1962, this 53-year old white male developed large pruritic erythematous plaques on his neck, upper arms, and thighs. In 1964, a diagnosis of lymphoma (mycosis fungoides) was made. Treatment with ultraviolet light was helpful and in 1967 erythromycin prescribed for secondary infection had a clearing effect on the eruption.

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The patient was first seen by me in 1968, at which time he was hospitalized because he had numerous papular and nodular lesions, as well as some tumours, on his trunk, arms and thighs. Lymphadenopathy was absent. There was no history of unusual exposure to chemicals.

Biopsy of both a plaque and a large nodule showed the definitive histological picture of mycosis fungoides. General medical studies, including a chest X-ray, bone marrow and lymphangiogram, revealed no evidence of other disease or internal involvement.

A course of oral demethylchlortetracycline (300 mg twice daily), begun in May 1968, led to disappearance of many of the small papules and marked reduction in size of the nodules and tumours within 3 weeks. One month later the skin showed only a few papules and post-inflammatory pigmentation at the site of the former large plaques. The demethylchlortetracycline was continued in decreasing dosage for 8 years. During this period the patient had but a few small papules. A biopsy of one of these confirmed the diagnosis of mycosis fungoides.

Two years after the treatment had been stopped the patient returned with numerous large papules and nodules on the chest, arms and thighs. A biopsy was read as mycosis fungoides, tumour stage. Demethylchlortetracycline was prescribed in a dose of 300 mg/day, while arrangements were made for hospitalization. Within 2 weeks, all of the lesions had undergone a dramatic involution of at least 50% in size. Hospitalization was cancelled and by 1 month the mycosis fungoides lesions had disappeared completely, leaving only post-inflammatory pigmentation.

The patient has continued to take the demethylchlortetracycline daily for the past year with no recurrence other than an occasional small papule. Topically the only medication used has been hydrocortisone lotion (0·5%). No nitrogen mustard, radiation or ultraviolet light therapy has been used at any time.

Case 2

In 1974, this 50-year old white male developed a pruritic scaling erythematous eruption on his trunk and legs. Examination 9 months later also revealed a sharply delineated, erythematous, mottled, atrophic, scaling, bilaterally symmetrical process in both axillae and covering the entire bathing trunk area. This had been present since the patient had been 10 years of age. A clinical diagnosis of poikiloderma vasculare atrophicans with an associated mycosis fungoides was made.

The patient was hospitalized. General medical studies as well as a chest X-ray and a G.I. series showed no evidence of internal involvement. There was no lymphadenopathy. Five biopsies (arms, axilla, back and groin) each gave a histological diagnosis of mycosis fungoides with the underlying poikiloderma being evident in the lesions from those areas.

A 10-day course of topical nitrogen mustard (10 mg in 60 cc of water freshly prepared and applied over the entire surface once a day) was associated with clinical improvement in the trunk lesions, but a florid white papulosquamous eruption appeared in the groin, superimposed on the poikiloderma. Prior to the topical nitrogen mustard this had been completely inapparent and asymptomatic. KOH examination revealed numerous hyphae and a diagnosis of tinea cruris was made. Griseofulvin, 500 mg twice daily, was prescribed. One month later the plaques had completely disappeared and a biopsy of the healed sites revealed no evidence of mycosis fungoides. The griseofulvin therapy was continued for 3 years, during which period the patient remained free of plaques.

The griseofulvin was stopped in May 1977 and within 6 months the patient had a widespread recurrence of mycosis fungoides lesions. Resumption of griseofulvin (500 mg/day) was associated with clearing within 1 month. The patient has remained clear and continues his daily griseofulvin. No nitrogen mustard treatments have been given following the 10 day course given initially. Topical treatment has been limited to clotrimazole and a steroid cream.

DISCUSSION

The remarkable result in Case 1 were first observed by chance when an intercurrent infection required systemic antibiotic therapy. The effects of the demethylchlortetracycline proved to be substantial. Initially, in 1968, a month's course of demethylchlortetracycline led to complete involution of the tumour stage of mycosis fungoides. Inasmuch as this stage is routinely associated with a high mortality (Groth, Molin & Thomsen, 1979), the patient was kept on a maintenance dose of demethylchlortetracycline for 8 years. At the end of this period the antibiotic was stopped. Two years later a widespread reappearance of nodules and tumours called for rehospitalization to stage the disease. However, this was cancelled when within 2 weeks of demethylchlortetracycline therapy the lesions had largely involuted. Within two more weeks they had essentially disappeared. The patient at this time remains on maintenance therapy and is essentially free of lesions.

The second patient showed a similar clearing of histologically proven mycosis fungoides, but in this instance in the plaque stage. The observations strongly suggest that griseofulvin was responsible for the clearing, although prior to the recognition of the occult tinea infection, a short 10-day course of topical nitrogen mustard was given. Under ordinary circumstances, nitrogen mustard would have to be used as a continuing maintenance dose at least once a week (Van Scott & Kalmanson, 1973; Molin, Thomsen & Volden, 1979). In my patient, no further nitrogen mustard was used. Furthermore, when the maintenance dose of griseofulvin was stopped after 3 years, the mycosis fungoides recurred within 6 months. Resumption of griseofulvin was associated with complete clearing within 1 month.

Although the action of the demethylchlortetracycline and the griseofulvin could be a metabolic one affecting the malignant cell of mycosis fungoides, this seems unlikely since use of these drugs in three other patients with mycosis fungoides led to no clinical change. A more attractive assumption is that these antibiotics eliminated specific chronic infections which had been sources of continuous antigen release. It was this persistent antigenaemia which in turn had triggered the lymphoproliferative process we identify as mycosis fungoides. My experience would suggest that this disease is a reaction pattern with multiple causes.

The idea that such chronic antigenic stimulation of the T cell may result in mycosis fungoides has been clearly espoused by Tan *et al.* (1974) and Tan (1976), and later by Prunieras & Flandrin (1977). Significantly, the epidermis possesses a known antigen trap, the Langerhans cell (Shelley & Juhlin, 1976), and Rowden *et al.* (1979) have pointed to its involvement in mycosis fungoides. Finally, Van der Loo and his colleagues (1979) have recently shown a C-type virus particle regularly present in the Langerhans cell of each of seven patients with mycosis fungoides. Their observations again stress the possibility of antigen persistence as a cause of mycosis fungoides.

Likewise, in epidemiological studies (Green *et al.*, 1979) 64% of the patients with mycosis fungoides had antecedent fungal or viral infections, with the possibility of long term antigen exposure. Individuals with unusual exposure to toxic chemicals also appear to have a higher incidence of mycosis fungoides.

Further studies are needed on the significance of these persistent antigens in the pathogenesis of mycosis fungoides. In any event, exploring for and eradicating causal antigens, whether they be infectious or toxic, gives the therapist a new approach and the patient new hope.

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Brief Communications

Erythema multiforme during treatment with mianserin—A case report

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A 38-year-old female patient with an affective disorder and thyrotoxicosis and taking amitriptyline, neomercazole and diazepam was admitted to the Psychiatric Unit twice at 6-monthly intervals to stabilize her mental state. Amitriptyline and diazepam were gradually withdrawn during the following 2-week period. Mianserin, 30 mg daily, was then introduced to her treatment but had to be discontinued on the fourth day, on both occasions, because of the appearance of widespread and severe lesions of erythema multiforme. Lesions on mucous membranes were not observed. The eruption resolved completely after mianserin withdrawal and systemic treatment with intramuscular triamcinolone.

The thyrotoxicosis was found to be under control clinically and biochemically. The patient has had similar psychiatric episodes of equal severity and without any skin complaints concurrently. The time relationships did not suggest that her other drugs were responsible.

Physical examination and routine laboratory investigations revealed no evidence of any other cause of erythema multiforme. Mianserin is a piperazine-azepine compound (Wheatley, 1975) belonging to a relatively new generation of antidepressant drugs which also have significant antihistaminic properties.

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Oral zinc sulphate in alopacia areata—a double blind trial

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In 1976 Wolowa and Jablonska reported that two patients with alopecia areata regrew their hair after treatment with oral zinc sulphate (Wolowa & Jablonska, 1976). A double blind trial was therefore undertaken to investigate the effect of treatment with zinc in this condition.

METHOD

Forty-two subjects with alopecia areata, alopecia totalis or alopecia universalis started the trial and all but four completed it. The mean duration of the disease was 5 years (range 3 months–43 years). The subjects were divided into pairs matched for (in order of importance) duration of the disease, history of atopy, extent of the disease and age and sex. One from each pair received the active drug (Zincomed capsules, 220 mg zinc sulphate) and the other received an identical placebo capsule in a double blind fashion. The patients were instructed to take one capsule twice daily after food for a period of 3 months. Before starting treatment, blood and hair, when present, were taken for determination of zinc levels by neutron activation analysis. The zinc estimations in hair and blood were repeated at the end of the trial.

RESULTS AND CONCLUSIONS

There was no improvement in the extent or activity of the disease in those people on the active drug compared with placebo either by objective or subjective assessment despite the fact that serum zinc levels and the zinc levels in hair were increased in the group on the active drug at the end of 3 months (Table 1). These results do not support the suggestion that oral therapy with zinc sulphate may be beneficial in alopecia areata.

TABLE 1. Zinc levels (μg/g dry weight) in hair and serum before and after 3 months treatment

	Pre-treatment		Post-treatment	
	Serum	Hair	Serum	Hair
Active	77·5	162·4	112·2	179·0
Placebo	72·4	158·2	76·8	161·6

ACKNOWLEDGMENTS

I am grateful for the co-operation of the consultants of the Skin Hospital in allowing me to study patients under their care and also for the zinc estimations which were performed by Dr A.Molokhia.

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Comment

THE SPECIFICITY OF EARLY CHANGES IN THE SKIN DURING CARCINOGENESIS

The recognition of early precancerous changes which are specific to carcinogenesis in the skin would be extremely helpful in the diagnosis of early malignant change and in shortening present skin painting carcinogenicity tests. The present article discusses the likelihood of this occurring in the light of our current knowledge.

Mouse skin painting tests have been used as a bioassay system for tobacco smoke constituents for several years and much of the information presently available on mouse epidermal cell kinetics and pathology has been derived from this source. The first successful bioassay of a suspected carcinogenic substance using an epithelial surface was that of Yamagiwa & Ichikawa (1916) with the application of coal tar to the ears of rabbits. Wynder, Graham & Croninger(1953), were the first to establish tobacco smoke condensate as being carcinogenic to laboratory animals. They used mouse and rabbit epithelial tissues. The conventional tumour formation tests using mouse skin cannot be described as 'short term'. The appearance of the first tumour after painting with a maximum tolerated dose of tobacco smoke condensate can be as long as 9 months from the commencement of the test (Bibby, 1977). Much of the efforts in the field of mouse skin painting have been directed to examining the specificity of early changes in skin carcinogenesis in order to try to speed up the tests. Single applications of carcinogens in mouse skin have been shown by several workers (Reller & Cooper, 1944; Iversen & Edelstein, 1952; Evensen, 1961; Bibby, 1977) to show transitory reduction in mitotic index followed by an increase at a later stage. In a study with nude mice, Iversen & Evensen (1962) demonstrated that polycyclic hydrocarbons decrease the number of epidermal cells labelled with tritiated thymidine and interpreted this depression as a block in mitosis and an inhibition of DNA synthesis. This reaction was observed after painting with carcinogens only and was considered to be a specific effect. Several other workers do not agree with this statement, however, and Elgjo (1968) after evaluation of mitotic rate, duration and count concluded that alterations provoked by single applications of tobacco smoke condensate were similar to those observed in simple regenerative reactions. Stripping procedures in humans (Pinkus, 1951), pig (Oehlert, Karase & Bertelmann, 1966) and mouse skin (Hennings & Elgjo, 1970) also result in a short inhibition of DNA synthesis followed by an increase in cell proliferation. It would seem unlikely, therefore, that initial cell kinetic changes specific to carcinogenesis would have been demonstrated in the early stages of tobacco smoke condensate studies as these condensates contain a considerable array of toxic materials in addition to their carcinogenic constituents. The ability to separate irritant responses from carcinogen responses is essential in order to evaluate the viability of mouse skin painting experiments in carcinogen testing and in diagnosis of malignant change.

Formation of chemically induced epidermal tumours (Bibby, 1977) is preceded by thickening of the epidermis. Epidermal thickening is caused by an increase in both the number and size of cells. These responses are dose related and occur after both carcinogen and non-carcinogenic irritant treatment. After the formation of papillomas the interfollicular epidermis between papillomata is indistinguishable from irritant treated epidermis. The labelling index with tritiated thymidine in the epidermis, however, is rapidly elevated following carcinogen treatment and remains elevated until

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tumour formation, whereas the labelling index after irritant treatment eventually returns to normal as the skin becomes more resistant to the irritant.

Another approach to examining the specificity of carcinogenesis is to make careful observation of morphological changes. This can be done by means of electron microscopy. Ultrastructural studies have provided much information on chemically induced epidermal tumours and in particular on disorganization of the junction between epidermis and dermis. Frei (1962) discovered defects in the basement membrane of epidermal tumours induced by topical application of 9,10-dimethyl 1,2 benzanthracene followed by repeated applications of croton oil. Tarin (1967) in an ultrastructural study of methylcholanthrene carcinogenesis in mouse skin described the sequence of changes which occurred in both the epidermis and the dermis but concluded that the most striking alterations occurred at the dermo-epidermal junction. Tarin (1968) examined the specificity of the ultrastructural changes during epidermal carcinogenesis by comparison with chemical irritant treatment. He concluded that the changes in the dermo-epidermal junction were directly related to the carcinogenic process and were not the result of non-specific irritation. These ultrastructural alterations can also be demonstrated in mouse skin after painting with tobacco smoke condensate (Bibby, 1977). The most striking of these effects were separation of individual cells and disturbance at the dermo-epidermal junction, accompanied by formation of epidermal pseudopodia. Alterations at the dermo-epidermal junction have also been shown in a variety of non-cancerous conditions. Epidermal pseudopodia have been detected in guinea-pig skin exposed to hydroxyanisoles (Riley & Seal, 1968; Seal, Riley & Inman, 1969) and in similarly treated hamster cheek pouch (Woods & Smith, 1969). Wound healing in human skin (Ross & Odland, 1968) and mouse skin (Tarin & Croft, 1970) is accompanied by epidermal penetration, and Cox (1969) has demonstrated pseudopodia formation in psoriasis. During mouse skin painting tests the short term changes demonstrated by electron microscopy again cannot be described as being specific for carcinogenesis as they can also be demonstrated in other conditions. The failure to demonstrate any specific precancerous cell kinetic or morphological changes makes the possibility of shortening the skin painting bioassay system very unlikely.

Even though skin painting bioassay systems are undoubtedly useful in carcinogenicity testing of cosmetics, detergents, etc., their relevance as a measure of human cancer in other sites must be open to doubt. The skin forms a protective layer and as such is not an ideal site for the testing of substances thought to be carcinogenic, for example, in the respiratory tract. Studies using radio-labelled polycyclic hydrocarbons have revealed that only a very small percentage of the test compound applied to the skin actually enters the tissue (Bibby, 1977; Bibby & Smith, 1977a). The total activity recovered from the tissues accounted for only 22% of that applied to the surface of the skin 24 h previously. Almost 69% of the activity remained unaccounted for even after assaying skin washes, faeces and cage washes. Much of the radioactivity extracted from the tissues was concentrated in the alimentary canal indicating the necessity to prevent test animals from licking the test substance from the test site.

Another and perhaps the most important aspect of the mouse skin painting system which must be considered in discussing its relevance to human cancer is the question of viral involvement. Kakefuda, Roberts & Suntzeff (1970) reported the presence of intra-cisternal A-type virus particles in chemically induced squamous cell carcinomas in Leaden strain C57L mouse skin. Similar observations were made in BALB/c mouse skin tumours induced by polycyclic hydrocarbon treatment (Bibby & Smith, 1975). A further study (Bibby & Smith, 1977b) has revealed the presence of C-type viruses budding from cell membranes in squamous cell carcinomas induced in BALB/c mouse skin by painting with tobacco smoke condensate. These results suggest a possible activation of an RNA oncogenic virus by chemical carcinogens. Since there is no evidence to suggest such an involvement in human cancer, this is an area which must be viewed with some care at present.

In conclusion it would seem that use of mouse skin painting as a test for carcinogenicity has its limitations. However, it has provided a great deal of basic information on the early stages of epidermal neoplasia. Examination of cell kinetics and ultrastructure have to date failed to reveal any early changes which are specific to malignancy in the skin so there appears to be no short cut to the early diagnosis of skin cancers and hence to their successful therapy.

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British Journal of Dermatology (1981) **104**, 489.

Book Reviews

Manual of Dermatology. J.P.CALLEN, M.A.STAWISKI and J.J.VOORHEES (1980) Chicago, London: Year Book Medical Publishers, Inc. Pp. 294, 199 Illus. Price £16.25.

This book, which is intended for use by medical students and primary care physicians, is based on a teaching manual developed for undergraduates at the University of Michigan. Its authors are well-known at home and abroad, and are particularly interested in teaching. Indeed, there is a most enthusiastic Foreword by Dr Walter Shelley, himself a great communicator. The book is very well-produced, readable and comprehensive. The illustrations in black and white are mainly good and helpful, and it is intended that the text should be read in conjunction with a colour atlas. Details of treatment of diseases, e.g. tuberculosis, are not always given in the appropriate places, and the Latin grammar leaves much to be desired. So far as prescribing is concerned, there is a gratifyingly small use of trade names; the dose of sulphapyridine for dermatitis herpetiformis is higher than usual. These are minor criticisms for this is a sensible, practical book likely to find much favour with its readers.

O.L.S.SCOTT

A Colour Atlas and Textbook of the Histopathology of Mycotic Diseases. FRANCIS W.CHANDLER, WILLIAM KAPLAN and LIBERO AJELLO (1980) London: Wolfe Medical Publications Limited. Pp. 333, 604 col: illus. Price £35.00

The identification of fungi in tissue is an important feature of diagnosis in medical mycology. However, although the organisms are relatively large and distinctive, their appearances are often unfamiliar. This new book on the histopathology of fungal diseases is a welcome contribution to an area where the literature has been sparse. The book is divided into two main sections, the first devoted to a brief description of each disease and, in particular, histopathological features, and the second a comprehensive colour atlas showing the organisms in tissue. The different stains, the techniques and role of immunofluorescence and the variants and aberrant forms of fungi are reviewed in detail. Considerable attention with illustrations has been paid to the confusing aspects of differential diagnosis such as the separation of Leishmania from Histoplasma. The book contains a useful glossary and bibliography.

The descriptive sections are brief and concentrate on the histopathology rather than the disease process, but the appearances of each organism, both *in vitro* and *in vivo* are compared. There are few cross references between the pictorial and descriptive sections. The terminology is straightforward although the authors use a few unfamiliar designations such as Histoplasmosis capsulati for small form or classical histoplasmosis. The colour illustrations are clear, apart from some of the mycetoma grains, and show the wide range of morphology exhibited in each disease. Much of the success of this book will depend on the quality of the reproduction of coloured photographs and by and large this has been excellent.

The book will be extremely useful to both pathologists and dermatologists particularly those working in areas where a wide range of subcutaneous or systemic mycoses occur. Although the authors have made it clear that there is little substitute for experience in this area, they have painted a comprehensive picture sufficient to guide most through the commonly encountered pitfalls in the recognition of fungi in tissue. The book is expensive at £35.00, but this reflects the large number of colour photographs it contains.

R.J.HAY

Biological Effects of Ultraviolet Radiation. WALTER HARM. IUPAB Biophysics Series (1980) Cambridge University Press. Pp. 216. Price £15.00 (hard cover), £4.95 (paperback).

In Photobiology, as in other advancing fields of study, there is a need for a regular gathering together of current knowledge and belief. This may be done in the form of annual reviews of various kinds or as proceedings of congresses. The International Union of Pure and Applied Biophysics has sponsored such a review in the form of a text book, just over 200 pages long, by Walter Harm, Professor of Biology at the University of Texas in Dallas, published in a pleasant format by Cambridge University Press. At the same time as setting out the various elementary principles of photobiology, Professor Harm presents a thorough treatment of the field at the molecular and microbiological levels. It is at these levels that his own original contributions, quite properly highly regarded, are concentrated. However, the effects of ultraviolet radiation on higher organism cells and on the skin are dealt with summarily. Nonetheless, this book is a useful addition to the specialist library, in effect, updating the excellent 'Molecular Photobiology' of Smith and Hanawalt. At £15.00 the book is too expensive to recommend for the general dermatological readership. Entry to the relevant literature may be better obtained through the Magnus opus 'Dermatological Photobiology' and also through the highly commendable 'Photo-medicine' chapters in recent editions of the Rook and Fitzpatrick textbooks.

B.E.JOHNSON

A Guide to Leprosy Control. World Health Organization (1980) 97 pages. Price Sw.fr.15.00. (French and Spanish edition in preparation.)

This is on the whole an excellent little book (90 pages), which is also to be translated into French and Spanish. It is not intended as a competitor to the standard textbooks on leprosy such as Bryceson & Pfaltzgraff: rather it is intended as an update to those involved in the treatment of leprosy, with official guidelines as to therapeutic regimes, etc. It comes at a crucial time, as it is now realised that dapsone resistance is already a major problem throughout the tropical world.

The sections in the book cover the following subjects: the leprosy problems in the world; present knowledge of the epidemiology of leprosy; the diagnosis, classification, treatment and prophylaxis of the disease; technical policy and strategy in leprosy control, and the results that may be expected from control projects; health education of the patients and the training of leprosy staff; medical, social and legal measures which pertain to the disease; established research and co-ordination schemes and suggested research projects to solve important problems which presently exist. There are also 6 annexes on the following subjects: preparation of standard integral lepromin; techniques of examination; suggested drug combinations; control of sulphone intake by urine tests; classification of disabilities; recording and reporting of leprosy patients.

The problems of dapsone resistance must not be underestimated. It is absolutely essential that patients with resistance to this drug be treated in the proper manner, otherwise patients will quickly develop resistance to the other few drugs efficaceous in this disease, and this is particularly likely to occur with rifampicin which is being handed out willy-nilly in several parts of the world. This booklet gives excellent advice on the avoidance of such problems and will be an essential buy for anyone working in the leprosy field.

R.STC.BARNETSON

The Physiology and Pathophysiology of the Skin. Edited by A. JARRETT (1980) London: Academic Press. Volume 6, 284 pp. Price £27.20.

This 6th volume in the series contains sections on the structure, chemistry and function of the mucosae, as well as on vitamin A and transepidermal water loss. Although dermatologists regard mucosae as a legitimate part of their domain, their understanding of the anatomy and physiology of mucosae doesn't match up to their clinical interest in these structures. For this reason the clearly and concisely written sections on the mucosae fill an obvious gap and are correspondingly useful. Katherine Grice's contributions on the significance and measurement of transepidermal water loss ought to be read by all dermatologists in training—then I may actually live to see the measurement made in dermatological practice. Dr Jarrett's sections on vitamin A are comprehensive and interesting but, sadly, are dated by not containing references to the newer retinoids. I like the general layout of the book. The indices are good and the referencing at the bottom of the page is a feature more books ought to adopt. The illustrations, in black and white, are copious and adequate in quality. Overall, I enjoyed this latest addition but, sadly, must warn that some of the material included is rather dated—particularly this applies to some of the referencing on histochemical observations. Nonetheless, I believe that we ought to be thankful for this contribution, as it contains topics that are sparsely covered elsewhere.

R. MARKS

Venereal Diseases. AMBROSE KING, CLAUDE NICOL and PHILIP RODIN (1980) London: Bailliere Tindell. Pp. 419, 190 figs, 5 tables, 15 col. plates. Price £14.00.

To have to review a textbook written by two of one's teachers and a colleague may be said to raise at least the possibility of special difficulties for the reviewer. In fact, no such difficulties arise as this, the fourth edition of 'Venereal Diseases' in 16 years, is, like its predecessors, everything a good textbook should be. It is well and authoritively written with scarcely a mistake or inaccuracy from cover to cover. It wastes no words whilst managing to cover all the important material and has really well chosen, up-to-date references at the end of each chapter. Finally, it is well bound and well illustrated with a good mix of black and white and colour photographs (why is it that colour reproductions are so expensive for medical texts and apparently cheap for pornographers—something to do with total sales volume and profits I suspect!). It also has an index which for once the terms 'accurate' and 'comprehensive' can be honestly employed. The price of £14.00 in hard back in 1981 is another important point in its favour and the publishers are to be congratulated.

Some people will feel that the problem of syphilis is over emphasized with 171 out of 403 pages being devoted to it, whilst two of the commonest reasons for patients presenting at a clinic in the U.K. namely, non-specific urogenital infection and psychological problems, achieve only 20 and 6 pages respectively. It is obviously important however; with the relatively low incidence of syphilis in many areas many modern doctors will be unfamiliar with the disorder and hence the need for a modern well illustrated account of the condition and it's management.

Reiter's Disease is well though briefly described and I would have liked to have seen a little more discussion of the problem of 'reactive arthritis' in a wider context, as, after all, it represents one of the most significant advances in our understanding at least something of one of the mechanisms of the causation of arthritis. I would also like to see the chapter on Public Health, excellent as it is, greatly expanded—the sexually transmitted diseases provide an almost perfect model for the demonstration of many of the techniques of epidemiology.

The chapters on gonorrhoea are completely up to date and especially so where the vital question of treatment is concerned. The tropical treponematoses, genital candidosis, genital ulceration, hepatitis and herpes are all clearly and briefly described and, where important, illustrated.

This book should be a standard purchase for the serious student and practitioner and of course all medical libraries. It represents excellent value for money.

J. K. BATES

British Journal of Dermatology (1981) **104**, 492.

News and Notices

Netherlands Dermatological Association

The next meeting of the Netherlands Dermatological Association will be held in the University of Nijmegen (Head—Professor J. Mali) on 16 May 1981.

Further information may be obtained from Dr S.M.C.Molhuysen-van der Walle, Secretary, Nederlandse Vereniging voor Dermatologie en Venereologie, Guido Gezellestraat 6, 1077 WP Amsterdam, The Netherlands.

Annual A.M.A.P.I. Symposium

This symposium, entitled 'Drugs and the Skin', will be held at the Royal College of Obstetricians and Gynaecologists, London on 4–5 June 1981. The following subject areas will be covered:

Advances in the understanding and treatment of psoriasis and acne.
Cutaneous adverse effects of drugs.
Novel cutaneous drug delivery systems.

For details and registration forms please write to: Mrs J. Wase-Bailey, A.M.A.P.I. Secretariat, 41 Queens Gate, London SW7 5HU.

Dermatopathology Foundation Course

A Dermatopathology Foundation Course in Gross and Microscopic Pathology of the skin will be held at Resorts International Hotel Casino, Atlantic City, New Jersey, 15–19 June 1981.

Forty hours credit AMA, Category 1.

Further information may be obtained from: The Dermatopathology Foundation, P.O. Box 377, Canton, Mass. 02021, U.S.A.

Dermatopathology Self-Assessment Workshop

A Dermatopathology Self-Assessment Workshop for Dermatologists, Pathologists and interested Physicians will be held at The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44106, on 10 October 1981. Further information may be obtained from the Course Director: Dr Wilma F. Bergfeld, M.D.

German Society for Dermatological Surgery

The 4th Annual Meeting of the German Society for Dermatological Surgery (Vereinigung für operative Dermatologie) will be held in Cologne, Germany, 12–14 June 1981.

Emphasis will be on developments in operative dermatology with special focus on the extremities. Topics will include nail surgery, warts, tumours, hyperthermic perfusion chemotherapy, veins and free communications. Further information may be obtained from: Prof. Dr Helmut Tritsch, Universitäts-Hautklinik, D-5000 Koeln 41, Germany.

Third International Congress of Dermatologic Surgery

The Third International Congress of Dermatologic Surgery will be held in Vienna (Austria), 11–14 October 1981.

Topics will include Flaps and Grafts, Vein Surgery, Oral Lesions, Haemangiomas, Aesthetic Surgery and Free Communications.

Further information may be obtained from: Dr E. Diem, C/o Wiener Medizinische Akademie, Alser Strasse 4, A-1090 Vienna, Austria.

History of Dermatology Club

The Eighth meeting of the History of Dermatology Club was held at Tiro-A-Segno on McDougall Street in New York on Sunday, 7 December , according to Lawrence Charles Parish, M.D., Philadelphia, President of the Club.

The Samuel J. Zakon Lectureship was given by Ervin Epstein, M.D. of Oakland, California and Harry L. Arnold, M.D. of Honolulu, Hawaii. This Shana Alexander-James Kirkpatrick type dialogue on Dermatology was introduced by Rees B. Rees, M.D., of Las Vegas, Nevada.

The first Samuel J. Zakon Award in the History of Dermatology was awarded to Dr Alan Lyell of Glasgow, Scotland for his paper 'Daniel Turner (1667–1740) LRCP London (1711) MD Honorary, Yale (1723). Surgeon, physician and pioneer dermatologist: the man seen in the pages of his book on the skin.' Honorable mention was given to Leon Goldman, M.D. of Cincinnati for his work 'Dermatology of the Bible' and to Julian Verbov, M.D. of Liverpool, England for his paper on 'Mummified Skin'.

Papers are now being called for the Second Annual Samuel J. Zakon Award in the History of Dermatology. This competition is open to all dermatologists in practice or in training. Manuscripts should be submitted to John Thorne Crissey, M.D., Chairman, Samuel J. Zakon Award Committee, 960 East Green Street, Pasadena, CA 91106, by 1 October 1981.

Both the Zakon Prize and the Zakon Award are given in memory of Dr Samuel J. Zakon, noted Chicago dermatologist and first historian of the American Academy of Dermatology.

ANNOUNCEMENT

Bill Reed International Travelling Fellowships

Three fellowships will be awarded this year as follows:

(1) The winner of the Poster Session at the Annual Meeting of the European Society for Dermatological Research will be awarded $800.00 to help support a trip to attend the Annual Meeting of the American Academy of Dermatology.

(2) The winner of the Stelwagon Prize for the best paper presented at the Resident's Forum at the Annual American Academy of Dermatology meeting will receive an $800.00 award to support a trip to Europe where the awardee will present a paper at the Annual Meeting of the British Association of Dermatologists in England. Further travel to European centres can also be arranged.

(3) A third award of $800.00 will be given to an academically oriented dermatologist in the *Private Practice of Clinical Dermatology* who has been out of training no more than five years. It is meant to support travel for educational purposes and to broaden contacts with dermatological investigators. Anyone interested in applying for this award should send their curriculum vitae and a covering letter indicating how they plan to spend the award funds to the Chairman of the Committee on International Affairs of the A.A.D. at the Academy Office, 820 Davis Street, Evanston, Illinois 60201. The deadline for submission is 1 September 1981. Supporting letters from abroad relevant to the planned travel are desirable. The award will be announced in December and will be based on the originality and productivity of the applicant as well as the meritoriousness of the travel plans for continued clinical investigation.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr R.H.Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldly names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 104, Number 4, April 1981

CONTENTS

PAGE

Clinical and Laboratory Investigations

Recurrent urticaria: clinical investigation of 330 patients …… 369
LENNART JUHLIN

Hepatitis B surface antigen deposition in the blood vessel walls of urticarial lesions in acute hepatitis B …… 383
H.A.M.NEUMANN, P.J.M.BERRETTY, S.C.C.REINDERS FOLMER AND R.H.CORMANE

Distribution and degradation of albumin in extensive skin disease …… 389
A.-M.WORM, E.TAANING, N.ROSSING, H.-H.PARVING AND O.J.CLEMMENSEN

Photosensitization of *Trichophyton* with 8-methoxypsoralen to UV-A light radiation …… 397
TAKESHI HORIO AND SHOHEI WATANABE

In vivo effects of PUVA on lymphocyte function …… 405
WARWICK L.MORISON, JOHN A.PARRISH, KURT J.BLOCH AND JOEL I.KRUGLER

Some factors affecting prognosis in pemphigus vulgaris and pemphigoid …… 415
J.A.SAVIN

Epidemiology of congenital pigmented naevi: II. Risk factors …… 421
EDUARDO E.CASTILLA, MARIA DA GRAÇA DUTRA AND IÊDA M. ORIOLI-PARREIRAS

Incorporation of D-(^{3}H)glucosamine into normal and psoriatic epidermal glycoconjugates …… 429
IAN A.KING, ANNE TABIOWO AND C.J.PAUL

Sulphhydryl and disulphide stainings of subepidermal hyaline bodies …… 437
KIICHIRO DANNO AND TAKESHI HORIO

Pharmacology and Treatment

Serum levels of trimethylpsoralen after oral administration …… 443
L.STOLK, A.H.SIDDIQUI AND R.H.CORMANE

Serum and saliva levels of 8-methoxypsoralen after rectal administration as a micro-enema …… 447
L.STOLK, A.H.SIDDIQUI, A.KAMMEYER, R.H.CORMANE AND P.A.VAN ZWIETEN

Inhibition of lipase production in *Propionibacterium acnes* by sub-minimal-inhibitory concentrations of tetracycline and erythromycin …… 453
G.F.WEBSTER, K.J.MCGINLEY AND J.J.LEYDEN

Case Reports

Zinc deficiency with transitory acrodermatitis enteropathica in a boy of low birth weight …… 459
INGA BLOM, STEN JAMESON, FREDRIK KROOK, BIRGITTA LARSSON-STYMNE AND LARS WRANNE

Acne induced by dantrolene …… 465
A.C.PEMBROKE, S.R.SAXENA, M.KATARIA AND K.D.ZILKHA

Richner-Hanhart syndrome (tyrosinaemia-II) (report of four cases without ocular involvement) …… 469
A.REHÁK, M.M.SELIM AND G.YADAV

Demethylchlortetracycline and griseofulvin as examples of specific treatment for mycosis fungoides …… 477
WALTER B.SHELLEY

Brief Communications

Erythema multiforme during treatment with mianserin—a case report …… 481
EHSANULLAH QURAISHY

Oral zinc sulphate in alopecia areata—a double blind trial …… 483
R.D.EAD

Comment

The specificity of early changes in the skin during carcinogenesis …… 485
M.C.BIBBY

Book Reviews …… 489

News and Notices …… 493

British Journal of Dermatology

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For the topical treatment of hyperhidrosis specifically involving the axillae, hands or feet.

DIRECTIONS FOR USE:
Apply to the affected sites at night as required and allow to dry. Wash off in the morning.

PRECAUTIONS:
Restrict application to affected sites only. Keep away from the eyes.

PACKAGE QUANTITY AND BASIC NHS PRICE:
10ml glass roll-on bottle £2.25.

REFERENCE:
British Medical Journal, 1978, **2**, 84.

PL 0173/0030

Anhydrol is a registered trade mark

Squeeze out a gel rub in a lotion.

Although presented in a tube, 'Synalar' Gel behaves, upon application, as an aqueous solution.

Containing a minimum of gelling agent, it becomes, under friction, a watery, greaseless liquid which rubs into the scalp easily and completely.

Yet, 'Synalar' Gel is more controllable than a true solution and therefore less wasteful; it is non-irritant and cools without excessive drying.

With all the advantages of a solution, and few of the disadvantages, 'Synalar' Gel provides a unique answer to the problems of scalp dermatoses.

Synalar gel
Fluocinolone acetonide
The solution in a tube.

Prescribing Notes

Presentation 'Synalar' Gel consists of Fluocinolone Acetonide BP 0.025% w/w, presented as a clear, water-miscible gel. **Uses** Specific indications for 'Synalar' Gel are seborrhoea, seborrhoeic dermatitis and psoriasis of the scalp. **Dosage and administration** A small quantity of 'Synalar' Gel is massaged into the scalp night and morning using the finger tips. **Contra-indications, warnings, etc.** 'Synalar' Gel is contraindicated in tuberculous, fungal, syphilitic and most viral lesions of the skin, and acne rosacea. Where there is a bacterial infection associated with an inflammatory skin condition, 'Synalar' should only be administered if adequate antibacterial cover is also given. Topical administration of corticosteroids to pregnant animals can cause abnormalities of fetal development. Although the relevance of this finding to human application has not been established, when topical steroid treatment is considered necessary during pregnancy both the amount applied and the length of treatment should be minimised. Long term continuous topical steroid therapy should be avoided since adrenal suppression can occur, particularly when infants are being treated. With 'Synalar' Gel side effects are extremely rare, but, as with all topical corticosteroids, the occasional patient may show an adverse reaction. **Product licence number** 0029/5039 **Basic NHS Price:** £1.15 (30g tube). 'Synalar' is a trade mark. Full prescribing information on all 'Synalar' preparations is available upon request.

ICI

Imperial Chemical Industries Limited,
Pharmaceuticals Division,
Alderley House, Alderley Park,
Macclesfield, Cheshire.

Synalar gel

Fluocinolone acetonide

The solution in a tube.

3675

for nurses

Dermatological Nursing and Therapy

R.H. Seville MD FRCP and
Eileen Martin SEN, *both*
of the Beaumont Hospital,
Lancaster

1981. 240 pages, 110 illustrations.
Paper, £7.50

This is a concise nursing text which classifies skin disorders and details the nursing therapy required. It covers all aspects of dermatological illnesses, from the physical to the psychosomatic, introducing both common and rare skin complaints and emphasises the importance of nursing management. Nurse–patient and doctor–patient relationships are stressed as not only complementary to but inseparable from scientific dermatology.

The text is abundantly illustrated with black and white and colour photographs and the authors' highly readable and informative text will make this book invaluable to all those involved with the care of patients suffering from skin disorders.

Emergency Procedures and First Aid for Nurses

Muriel Skeet
July '81. 256 pages, 114 illustrations.
Paper, about £4.50

Blackwell Scientific Publications

The gentle touch

When treating eczema in delicate skin areas, it is important to use a topical steroid which is gentle on the skin. Hydrocortisone is safe but not fast acting. The condition needs to be resolved quickly to lessen the risk of secondary infection from scratching. Locoid is the answer, because it works faster and more effectively than hydrocortisone. And although it is as strong and as fast-acting as the potent fluorinated steroids (1, 2, 3), clinical and experimental studies have shown it to have a lower capacity for side effects (4, 5, 6).' Locoid is the logical choice for effective treatment with a wide margin of clinical safety.

Dosage: Apply two to four times daily.

Contra-Indications: In the presence of infection unless used with suitable chemotherapy. Should not be used in large amounts or for long periods in pregnancy. Avoid long-term continuous therapy in infants.
Locoid is a trademark for hydrocortisone 17-butyrate.
P/L nos.: 0166/0058; 0166/0059; 0166/0060.
30g cream/ointment £1.73. 20ml lotion £1.85.

References 1. **Dermatologica 152** (Suppl. 1) 209-214 (1976). 2. **Brit. J. Clin. Pract. 27**, No. 5 (1973) 3. **Brit. J. Clin. Pract. 26**, No. 6 (1972). 4. **Brit. J. Derm. 95**, 566 (1976) 5. **Brit. J. Clin. Pract. 32**, 345 (1978). 6. **Dermatologica 160**, 261-269 (1980).

Locoid

Hydrocortisone 17-butyrate 0.1%

RELY ON ITS STRENGTH
TRUST IN ITS SAFETY

Brocades|Great Britain|Ltd.

West Byfleet, Weybridge, Surrey, KT14 6RA
Telephone: Byfleet 45536/42291

Our Application is often successful, even in difficult cases

Dermovate Scalp Application has been specially developed for difficult, stubborn scalp dermatoses. The preparation, contained in a plastic squeeze bottle with an elongated nozzle, is easily applied through the hair direct to the scalp where it spreads evenly over the lesions – with no mess or inconvenience.

Dermovate Scalp Application is odourless and leaves no visible residue on the hair. The alcohol/water base dries quickly producing an immediate cooling effect accompanied by relief from itching and inflammation. Twice daily application is often successful in rapidly clearing the lesions, even in difficult cases.

Dermovate

(clobetasol propionate)

Scalp Application

Prescribing information

Uses

Psoriasis and recalcitrant eczemas of the scalp. Clobetasol propionate is a highly-active topical corticosteroid which is indicated for use in short courses for conditions which do not respond satisfactorily to less active steroids.

Dosage and administration

Apply sparingly to the scalp night and morning until improvement occurs. As with other highly-active topical steroid preparations, therapy should be discontinued when control is achieved. Repeated short courses of Dermovate Scalp Application may be used to control exacerbations. If continuous steroid treatment is necessary, a less potent preparation should be used.

Contra-indications

Infections of the scalp. Hypersensitivity to the preparation.

Precautions

Care must be taken to keep the preparation away from the eyes. Do not use near a naked flame. Long-term continuous therapy with Dermovate Scalp Application should be

Development of secondary infection requires withdrawal of topical corticosteroid therapy and institution of appropriate systemic antimicrobial therapy.

Topical administration of corticosteroids to pregnant animals can cause abnormalities of fetal development.

In general, topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

Dermovate preparations are usually well tolerated, but if signs of hypersensitivity appear, application should be stopped immediately. As with other topical corticosteroids, local atrophy may result from prolonged treatment, particularly if occlusive dressings are used. When extensive areas are treated, sufficient systemic absorption may occur to produce the features of hypercorticism, especially in infants and children. This effect is more likely to occur after prolonged treatment, particularly if occlusive dressings are used. In rare instances, treatment of psoriasis with

Presentation

Plastic squeeze bottle with elongated nozzle containing 25 or 100ml

Basic NHS cost (exclusive of VAT)

25ml bottle £2·72

100ml bottle £9·65

Product Licence number

4/0242

Glaxo

Leaders in topical steroid therapy

Further information on Dermovate (trade mark) Scalp

In eczema and dermatitis

METOSYN

Fluocinonide Trade Mark

GETS THERE FIRST

The short-course
steroid for wet
or dry skin lesions.

Full prescribing information is available on request from
Stuart Pharmaceuticals Limited
Carr House, Carrs Road, Cheadle, Cheshire SK8 2EG

STUART

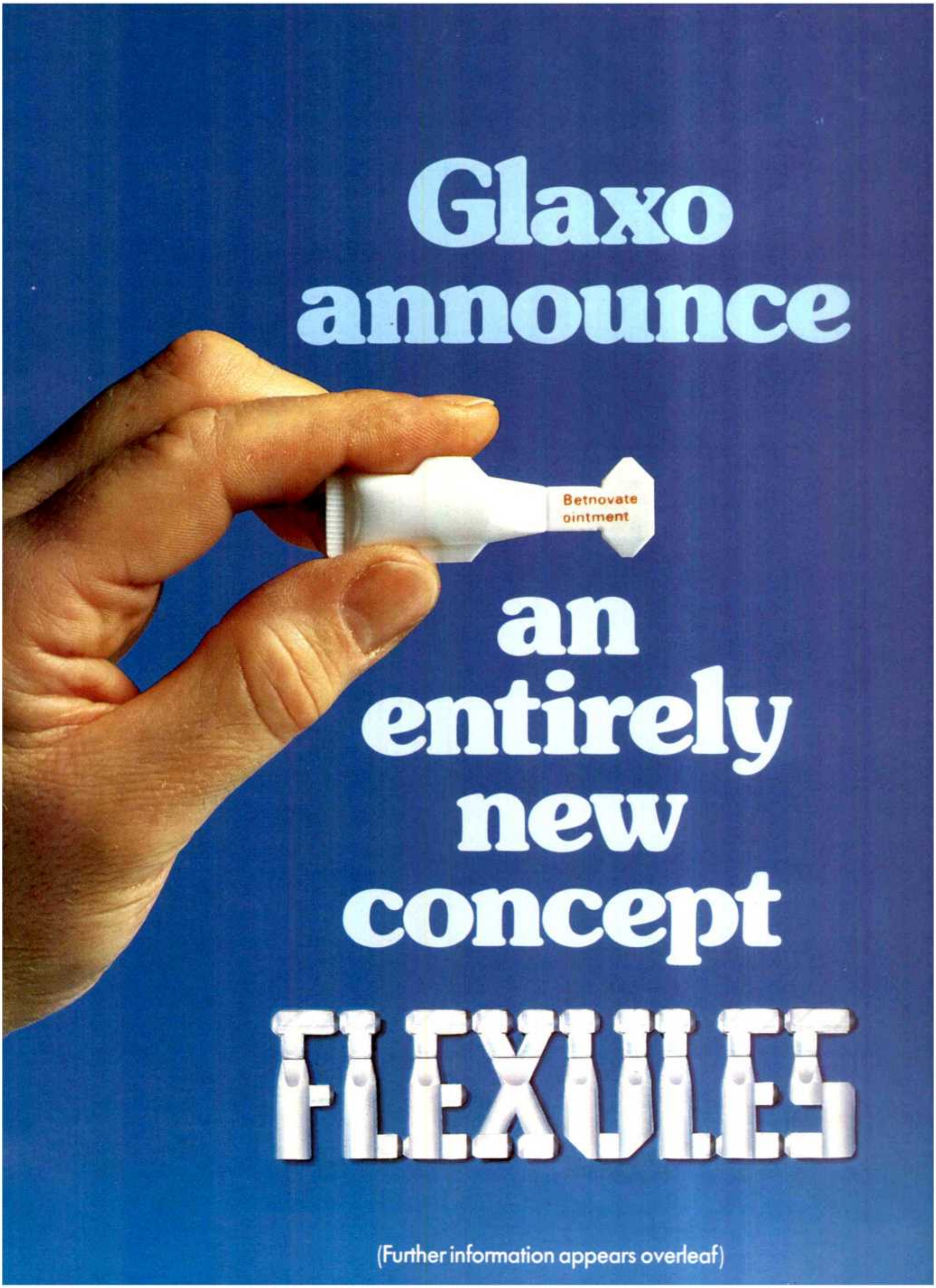
PUVA for Psoriasis

PUVA facilities for the treatment of psoriasis are now available to private patients who are referred by Consultant Dermatologists. The Waldmann PUVA 6000 unit, widely acknowledged as the best of its kind, is used. It allows wholebody treatment in the standing position. There are also Waldmann PUVA 180, 200 and 500 units for the treatment of hands, feet and other small areas.

A regime has been developed based on a London Teaching Hospital's experience and practice. The staff are professionally qualified and trained in the Photochemotherapy of psoriasis. Visits by Dermatologists are welcome by appointment.

An Honorary Medical Advisory Committee made up of four consultant dermatologists is supervising the professional standards and practice of the clinic. Dermatologists, who would like to know more about this facility, are asked in the first instance to contact:

The Physician-in-Charge,
Psoriasis UVA (Medical Referals Only) Ltd.,
20 Harley Street, LONDON W.1.
Telephone 01-580 0875



Glaxo announce an entirely new concept

FLEXULES

(Further information appears overleaf)

New from Glaxo

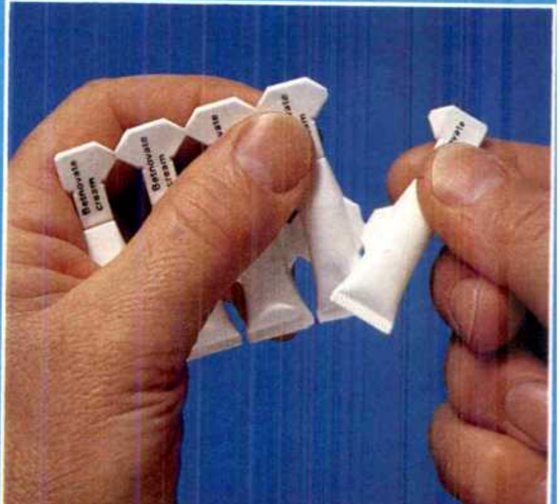
1 gram single applications of topical steroid creams and ointments.
In packs of 20.

Betnovate Flexules
(betamethasone valerate)
Cream and Ointment

Dermovate Flexules
(clobetasol propionate)
Cream and Ointment

Eumovate Flexules
(clobetasone butyrate)
Cream and Ointment

Efcortelan 1% Flexules
(hydrocortisone 1%)
Cream and Ointment



The excellence of the Glaxo topical steroids in the treatment of steroid-responsive dermatoses has been demonstrated in wide clinical use.
Flexules provide units-of-use single applications adding a new dimension of control. In addition to specifying the base and steroid appropriate to the condition you can now be more precise about the quantity applied and the duration of the course. And at the same time reduce contamination and wastage.
Each 1 gram Flexule can generally be regarded as being sufficient to cover lesions approximately the area of an adult forearm.

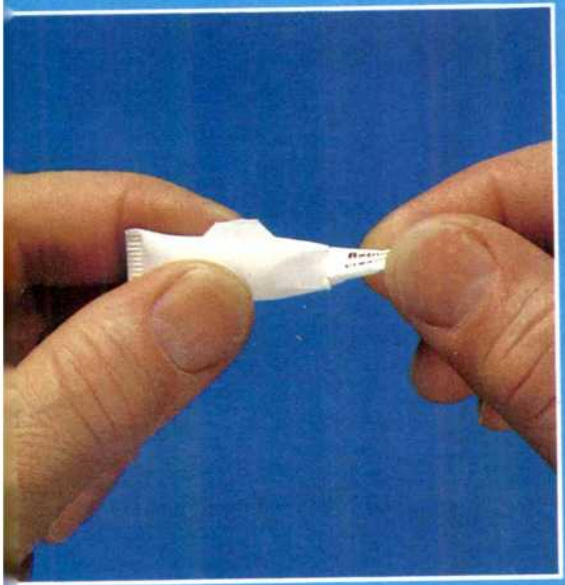
Prescribing information

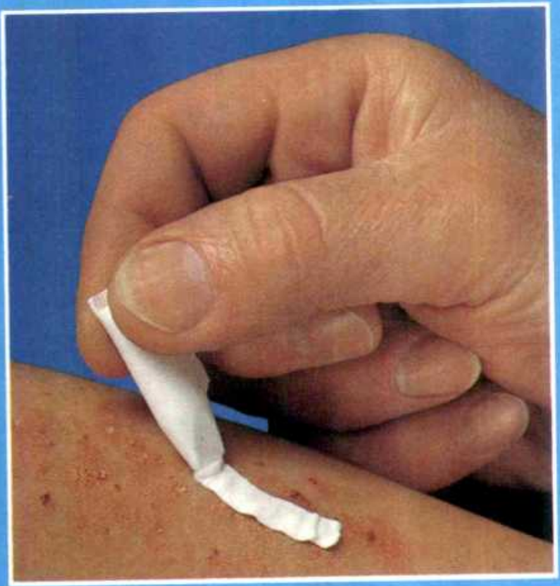
Dosage and administration
Sufficient cream or ointment should be applied to leave the affected area looking glossy. This should be applied up to three times daily depending on the circumstances of the case. However, with Dermovate once or twice daily application is sufficient.

Side effects
General:
With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also, prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism. This is more likely to occur in infants and children, and with occlusion. In infants the napkin may act as an occlusive dressing. Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.
In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.
Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Precautions
Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit

A new measure of control in topical steroid therapy





n comparison with tube presentations:

Flexules offer the physician

- Greater therapeutic control
- Improved prescribing precision
- More specific patient counselling
- Easier monitoring of patient compliance

Flexules offer the patient

- Treatment that is easy to understand and simple to use
- A convenient compact presentation, hygienic and easy to carry around

Glaxo Leadership and experience in topical steroid therapy

atrophic changes after prolonged treatment with potent topical corticosteroids.

Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy.

With all corticosteroids, prolonged application to the face is undesirable.

Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications

Bacterial, fungal or viral diseases of the skin. Dermovate and Betnovate are also contra-indicated for rosacea, acne and peri-oral dermatitis.

Hypersensitivity to the preparation.

Basic NHS cost (exclusive of VAT)	**Product Licence numbers**
Betnovate Cream/Ointment Flexules 20 × 1 gram £3·62	4/5121, 4/5124
Dermovate Cream/Ointment Flexules 20 × 1 gram £4·22	4/0219, 4/0220
Eumovate Cream/Ointment Flexules 20 × 1 gram £3·91	4/0233, 4/0254
Efcortelan Cream/Ointment 1% Flexules 20 × 1 gram £3·19	4/5081, 4/5086

Betnovate, Dermovate, Eumovate, Efcortelan and Flexules are trade marks. Further information is available from:
Glaxo Laboratories Limited, Greenford, Middlesex UB6 0HE

Why mess about with topical antifungals

...when oral Grisovin can be working from within?

For fungal infections, topical therapy is often both messy and inconvenient – and is unlikely to be as completely effective as oral Grisovin.

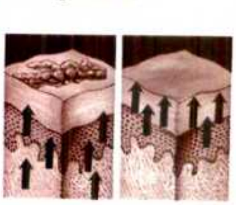
Grisovin is carried in the bloodstream to the basal layer and gets right to the root of ringworm infections. It protects the newly formed keratin cells from infection and as they move outwards, the fungus is expelled.

During the past 20 years, Grisovin has proved itself in clinical use to be the more effective therapy especially in long established ringworm infections. In addition its oral administration, two 500mg tablets a day, and its relative lack of side effects facilitates patient compliance.

Why mess about with topical antifungals when Grisovin gets right to the root of the problem?

GRISOVIN

griseofulvin



THE INSIDE STORY ON FUNGAL INFECTION

Prescribing information

Dosage and administration

Doses should be taken after meals for maximum absorption.

Adults: normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily. *Children:* usually 10mg/kg (5mg/lb) bodyweight daily in divided doses.

Duration of treatment: for hair or skin at least four weeks' treatment is required, whereas finger or toe nails may need six to twelve months' treatment. Continue for at least two weeks after all signs of infection have disappeared.

Contra-indications

Porphyria or severe liver disease.

Griseofulvin may cause deterioration in some patients with liver disease and liver function should be monitored in such conditions.

Precautions

Griseofulvin may decrease the effect of coumarin anticoagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established, but use during pregnancy is best avoided.

Side effects

Headache, and gastric discomfort sometimes occur, but usually disappear as treatment continues. Rarely, urticarial reactions and erythematous rashes have been reported.

Further information

Grisovin is not effective in infections caused by *Candida albicans* (monilia), Aspergilli, *Malassezia furfur (Pityriasis versicolor)* and Nocardia species.

Basic NHS cost	**Product Licence numbers**
(exclusive of VAT) £9·80	
Bottle of 100 tablets (500mg)	125mg: 4/5060 500mg: 4/5061

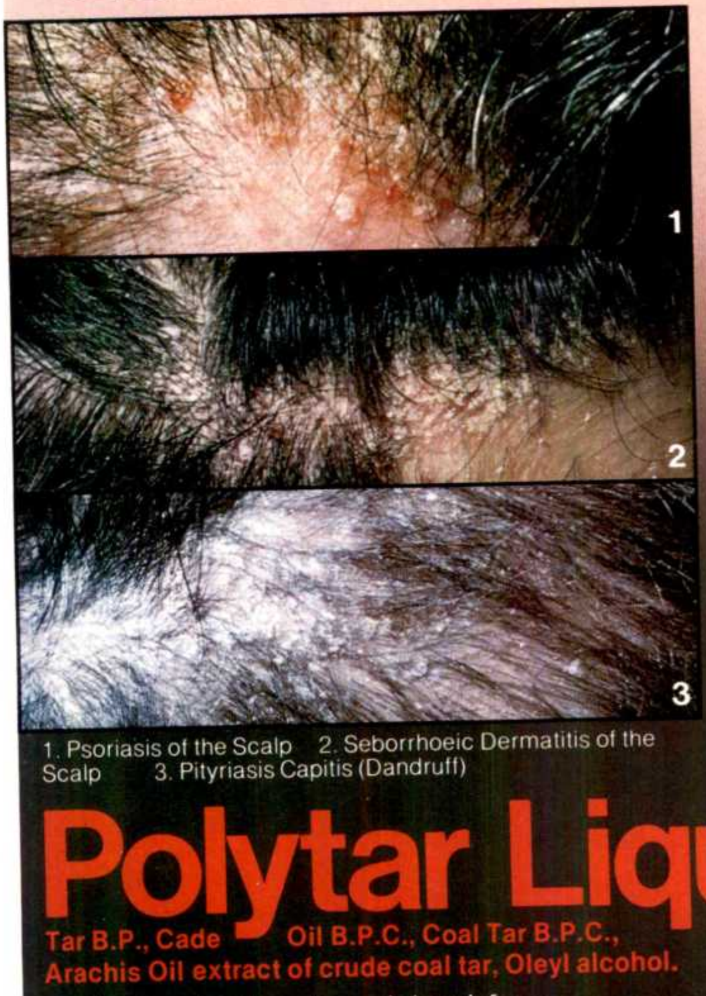
Glaxo

Full prescribing information on Grisovin (trade mark) is available from:

Glaxo Laboratories Limited
Greenford, Middlesex UB6 0HE

Common Disorders of the Scalp.

They all have one thing in common — the treatment



1. Psoriasis of the Scalp 2. Seborrhoeic Dermatitis of the Scalp 3. Pityriasis Capitis (Dandruff)

Polytar Liquid

Tar B.P., Cade Oil B.P.C., Coal Tar B.P.C., Arachis Oil extract of crude coal tar, Oleyl alcohol.

Acknowledged as ideal for long-term control.

NEW Polytar Plus with protein

Prescribing Information.

Presentation Polytar Liquid is a concentrated, antiseptic tar-medicated scalp cleanser containing the following active ingredients:

Tar B.P.	0.3%
Cade Oil B.P.C.	0.3%
Coal Tar B.P.C.	0.1%
Arachis Oil extract of crude coal tar	0.3%
Oleyl alcohol	1.00%

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
Polytar Liquid	65ml	57p
	150ml	82p
	350ml	£1.48
	1 litre	£3.90
Polytar Plus	150ml	90p

Product Licence Number
Polytar Liquid PL0174/5016 Polytar Plus PL0174/0037

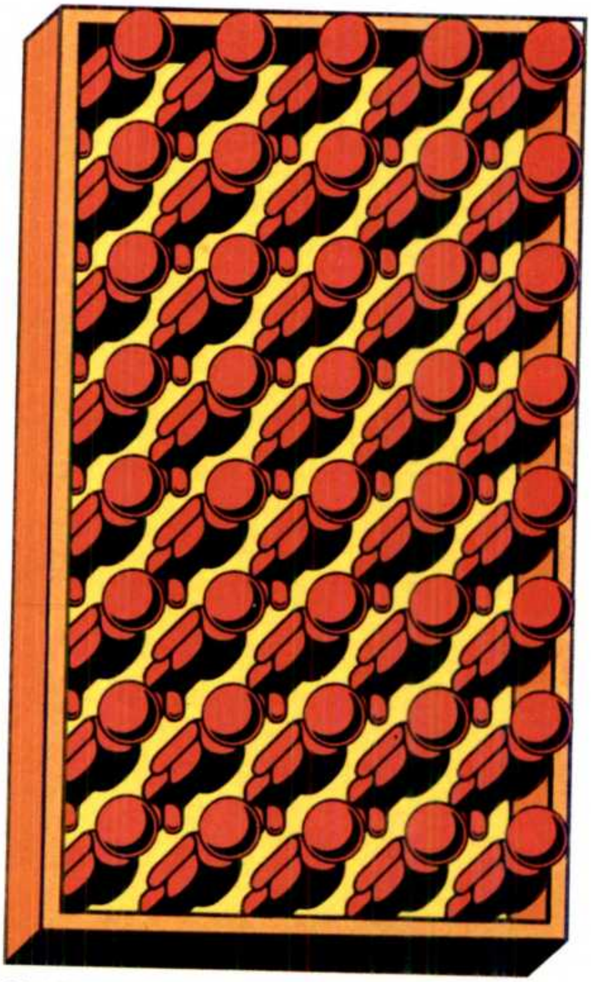
Full information and Data sheet on request.

Stiefel Laboratories (UK) Limited
Wellcroft Road, Slough SL1 4AQ

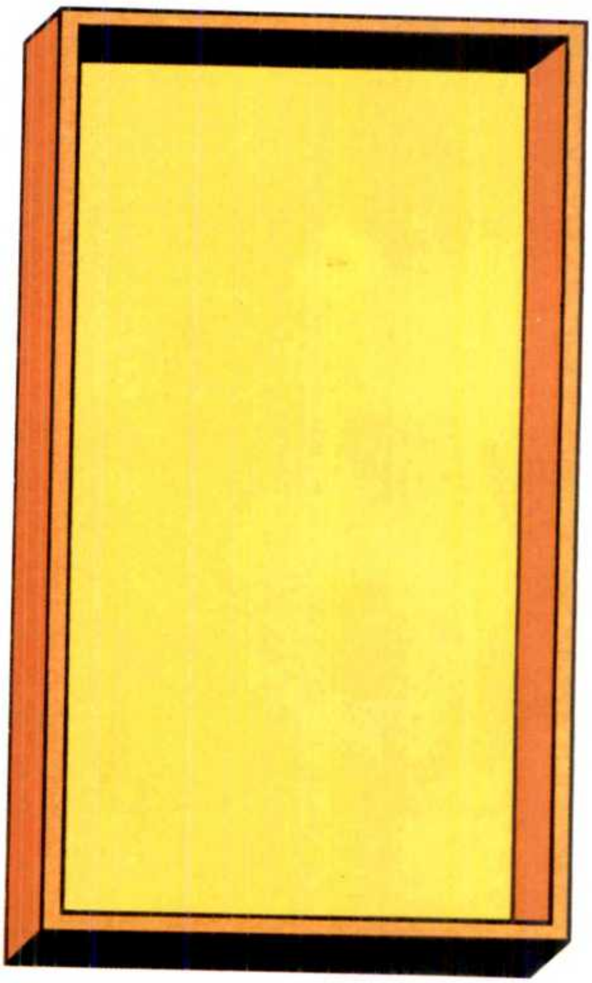
Dithrocream

dithranol BP

Natural selectioı !



All 40 patients in the trial preferred Dithrocream



Not a single patient preferred dithranol ointment

In a recent clinical trial,[1] Dithrocream 0.25% was compared with dithranol ointment of equivalent strength in the treatment of psoriasis.

Whilst both preparations were effective, there was a unanimous patient preference for Dithrocream with all 40 patients selecting the vanishing cream formulation.

Combining effectiveness, convenience and stability, Dithrocream is the patient's choice for psoriasis treatment.

Presentation

Dithrocream 0.1%: pale yellow aqueous cream containing 0.1% Dithranol B.P.
Dithrocream 0.25%: pale yellow aqueous cream containing 0.25% Dithranol B.P.
Dithrocream Forte: pale yellow aqueous cream containing 0.5% Dithranol B.P.

Indications

For the topical treatment of subacute and chronic psoriasis including psoriasis of the scalp. Dithrocream Forte should only be used for resistant cases.

Directions for use

Apply sparingly to the psoriatic lesions preferably at night and rub in gently and carefully until absorbed. Bathe before the next application. For scalp lesions, shampoo before use. Continue treatment until the skin is smooth and clear.

Contraindications

Acute psoriasis; dithranol sensitivity.

Precautions

Keep away from the eyes. Apply to psoriatic areas only. Use cautiously after recent application of potent steroids. Wash the hands after use.

Basic N.H.S. prices:

Dithrocream 0.1% 50gm tubes £2.10
Dithrocream 0.25% 50gm tubes £2.27
Dithrocream Forte 50gm tubes £2.54

Product Licence Nos.

Dithrocream 0.1%	PL 0173/0029
Dithrocream 0.25%	PL 0173/0028
Dithrocream Forte (0.5%)	PL 0173/0027

References:

1. *British Journal of Dermatology*, 1979, **100**, 475

Full prescribing information is available from
Dermal Laboratories Limited,
Gosmore, Hitchin, Hertfordshire SG4 7QR.

Dithrocream is a registered trade mark.

Now available in three strengths

British Journal of Dermatology (1981) **104**, 495.

Clinical and Laboratory Investigations

Discoid lupus erythematosus-like lesions and stomatitis in female carriers of X-linked chronic granulomatous disease

FLEMMING BRANDRUP, CHRISTIAN KOCH, MICHAEL PETRI,
MORTEN SCHIØDT AND KIRSTEN STÆHR JOHANSEN

Department of Dermatology, Gentofte Hospital and The Finsen Institute, Copenhagen;
Departments of Pediatrics TG, Rigshospitalet, Copenhagen; Department of Pathology, Sundby Hospital,
Copenhagen; Department of Oral Medicine & Oral Surgery, Rigshospitalet and Department of
Oral Pathology, Royal Dental College, Copenhagen, and Department of Clinical Microbiology,
Seruminstitutet, Rigshospitalet, Copenhagen, Denmark

Accepted for publication 18 July 1980

SUMMARY

The skin and oral mucosa were studied in an unselected series of carriers of X-linked chronic granulomatous disease, a hereditary condition in which phagocytic cells display a pronounced functional defect.

Three carriers had discoid lupus erythematosus (DLE)-like skin lesions which histopathologically were consistent with DLE of the hypertrophic and profundus type. Four patients had experienced photosensitivity in childhood. Seven patients had recurrent aphthous-like stomatitis which should be distinguished from the recurrent aphthous stomatitis seen in otherwise healthy individuals.

The remarkably high incidence of DLE-like symptoms in heterozygous carriers might be related to the presence of mixed populations of defective and normal phagocytes. The variable expression of skin symptoms may be related to uneven distribution of abnormal to normal phagocytes.

Female patients with these clinical symptoms, especially the combination of DLE-like skin lesions and aphthous-like stomatitis, should be suspected of being carriers of chronic granulomatous disease and studies of phagocyte function *in vitro* should be performed, since the diagnosis of the carrier state is of utmost importance for genetic counselling before pregnancy.

Chronic granulomatous disease (CGD) is a hereditary condition in which polymorphonuclear leukocytes (PMN) display a pronounced bactericidal defect (Quie, 1975) caused by a derangement in the normal oxidative metabolism of these cells. Most reported cases have involved boys (Johnston & Newmann, 1977) in whom the disease is inherited as an X-linked recessive trait (Windhorst *et al.*, 1968; Quie, 1975). Affected children suffer from severe recurrent and chronic infections caused by

Correspondence: Dr Flemming Brandrup, Department of Dermatology, Odense University Hospital, DK-5000 Odense C, Denmark.

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catalase-positive bacteria and fungi (Johnston & Newmann, 1977). Skin infections, eczematous reactions (Windhorst & Good, 1971), suppurative lymphadenitis, otitis and stomatitis (Wysocki & Brooke, 1978) may be seen in early life, and very often more severe infections like pneumonia, osteomyelitis, abdominal abscesses, meningitis and septicaemia may occur.

PMN from the heterozygous female carriers of the X-linked form display a partial defect in oxidative metabolism and bacterial killing (Windhorst *et al.*, 1968). Studies of the ability of individual PMN from CGD homozygotes and heterozygotes to reduce nitroblue tetrazolium (NBT), a function of normal oxidative metabolism, reveal mixed populations of normal and defective cells (Windhorst *et al.*, 1968). This finding is in agreement with the Lyon hypothesis of random X-chromosome inactivation (Lyon, 1962). However, most carriers are free of undue infections, but skin diseases including discoid lupus erythematosus (DLE) (Landing & Shirkey, 1957; Douglas, Davis & Fudenberg, 1969; Klebanoff & White, 1969; Thompson & Soothill, 1970; Schaller, 1972; Humbert *et al.*, 1976) and Jessner's lymphocytic infiltration of the skin (MacFarlane, Speirs & Sommerville, 1967; Nelson, Dahl & Goltz, 1977) have been reported. Recurrent aphthous stomatitis has also been reported in these carriers (Windhorst *et al.*, 1968; Douglas, Davis & Fudenberg, 1969; Schaller, 1972).

In order to describe in greater detail the clinicopathological findings and their frequency of expression in the skin and oral mucosa, we have undertaken a prospective study of nine unselected known carriers of X-linked CGD.

MATERIAL AND METHODS

Eight mothers, one sister and a maternal aunt of boys with CGD, representing all who could be contacted through the Danish registry for immunodeficiencies, were studied.

The carrier state was established by functional tests on peripheral blood PMN *in vitro*, showing decreased intracellular killing of *Staph. aureus* (Koch, Søgaard & Christensen, 1973) and decreased stimulated oxidative metabolism as judged by hexose monophosphate shunt activity, chemiluminescence, and reduction of NBT by standard techniques. By all parameters the carriers demonstrated a partial defect, resulting in intermediate values between those of affected boys and normal controls.

In three cases punch biopsies were taken from skin lesions for histopathological examination, and in two cases direct immunofluorescence study was performed with fluorescein isothiocyanate-conjugated anti-human IgG, IgA, IgM, anti-fibrinogen and complement C_3. In two cases biopsies taken previously were reviewed.

The oral mucosa was examined clinically, histopathologically and immunopathologically during attacks of stomatitis in four patients (case 1–3 and 5) and clinically in two patients (case 4 and 9). Biopsies from affected as well as unaffected oral mucosa were divided, one half being processed for routine microscopy, the other half processed for direct immunofluorescence (IF) studies according to a method described previously (Schiødt *et al.*, 1974) with antisera for the presence of fibrinogen, IgG, IgA, IgM and complement C_3.

CASE REPORTS

Case 1

A.J., 42-year-old woman with a healthy daughter and a son with CGD. The patient had from the age of 7 years suffered from recurrent aphthous-like stomatitis with intervals of weeks or a few months. From 36 years of age recurrent hidradenitis in the axillae and genitofemoral region was present, and a sterile abscess in the buttock had been drained. From 30 years of age infiltrated, bluish red, slightly scaling small plaques had been present on the dorsa of the hands (Fig. 1), in the sternal region and periodically on the cheeks and nose. Following some previous lesions, slightly depressed

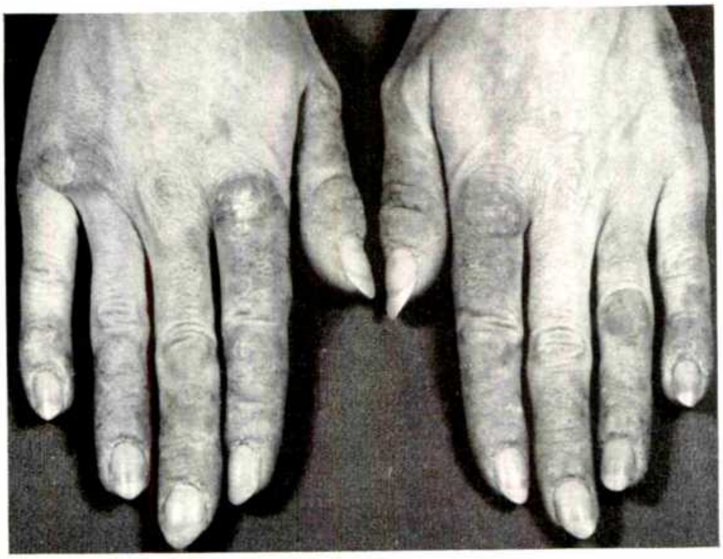

FIGURE 1. Case 1. Infiltrated plaques on the hands.

hypopigmented scars were seen. On the tips of the toes deep-set, partly haemorrhagic vesicles were seen. During treatment with hydroxychloroquine (Ercoquin) 250 mg twice a day the skin changes almost cleared, the stomatitis decreased in intensity and the condition is now controlled on 250 mg daily. Skin biopsies had been taken 1 year and 5 years before treatment.

Case 2

E.L.O., 36-year-old mother to a boy with CGD. The patient had suffered from photodermatitis in the summer since early childhood, but the photosensitivity has decreased in adult life. From 14 years of age recurrent aphthous-like stomatitis had been present at intervals of weeks or a few months. From the age of 24 recurrent eruptions of red, infiltrated lesions on the chin, cheeks, and forehead had been present, often with rosacea-like papules (Fig. 2). On both palms dark red, well demarcated nummular lesions were seen (Fig. 3). On the tips of some fingers and toes deep-set vesicles and slight scaling were present. During treatment with hydroxychloroquine (Ercoquin) 250 mg twice a day the skin changes almost cleared, and the stomatitis decreased in severity after 1 month. The condition is controlled on 250 mg daily. One skin biopsy was taken before treatment.

Case 3

E.F.C., 28-year-old woman, sister to two boys who died from CGD. Another sister (case 6) had a son with CGD. The patient had from the age of 7 years suffered from recurrent stomatitis at intervals of weeks. From 14 years of age red, infiltrated, slightly scaling lesions were seen on the hands and fingers and periodically over the shoulders. Typical perniosis of fingers and toes was present. Deep-set haemorrhagic vesicles and slight scaling were seen on the tips of the big toes. A skin biopsy had been taken 5 years previously and new biopsies were taken.

Case 4

P.S.J., 32-year-old mother to a boy with CGD. The patient suffered in childhood from polymorphous light eruption during spring and summer. She was examined at the Finsen Institute at the age of

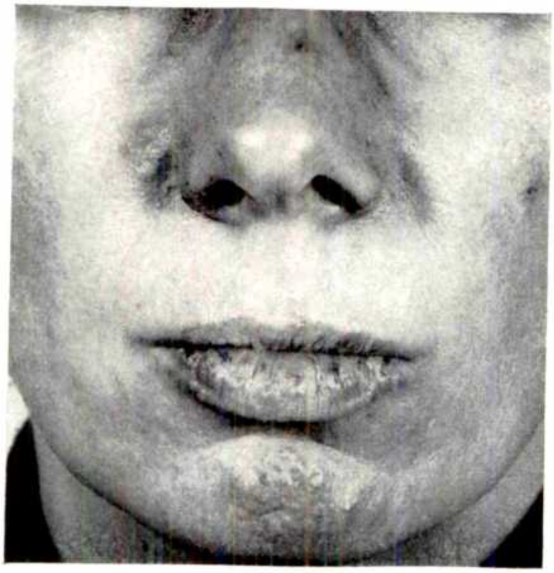

FIGURE 2. Case 2. Bluish-red rosacea-like infiltrates on the face.

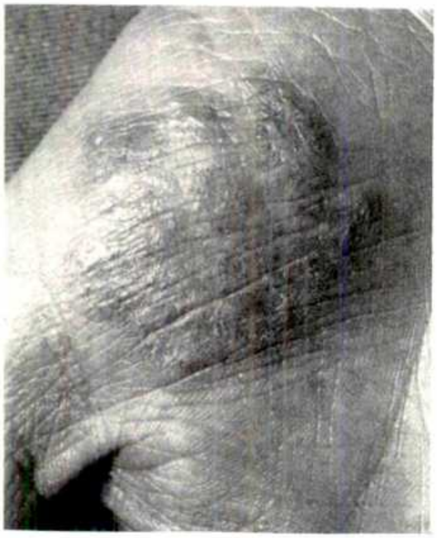

FIGURE 3. Case 2. Well-demarcated dark red patch on right palm.

7 years, when she presented with photodermatitis of the cheeks. At the present examination the light sensitivity had decreased, and the skin is now normal except for slight comedo acne. For many years the patient has suffered from recurrent stomatitis about once a month.

Cases 5–9

The findings in these cases are summarized in Table 1. One of these mothers mentioned photodermatitis in childhood and three suffered from recurrent aphthous-like stomatitis. Three patients had been seen by dermatologists because of hand eczema classified as irritant eczema. One patient had acute glomerulonephritis with a typical course at the age of 11 years, but no sequelae. One patient had been treated for sterile subcutaneous abscesses in the breast.

TABLE 1. Clinico-pathological findings in female carriers of CGD

Case		Age yr	Photo-sensitivity	DLE-like skin changes	LE serology	Recurrent stomatitis	Abscesses Hidradenitis	Hand eczema	Associated findings
1	AJ	42		+(30)	neg	+(7)	+(36)		
2	ELO	36	+(1)	+(24)	neg	+(4)	+		Sequelae of chorioretinitis in right eye
3	EFC	29	+(14)	+(14)	neg	+(7)			
4	PSJ	32	+(EC)		neg	+(C)			
5	JRJ	38	+(C)		neg	+(23)	+	+	Acute glom.nephr. (11). No seqv.
6	TFN	25			NT	+(C)			
7	BR	42			NT			+	
8	KL	27			NT				
9	JBL	45			NT	+		+	

NT = not tested; ( ) = estimated age at onset; EC = early childhood; C = childhood.

RESULTS

Laboratory findings

In all cases the heterozygous carrier state was established by granulocyte function tests as mentioned previously. In five cases tested no antinuclear antibodies, anti-DNA-antibodies or LE cells were found, and the Latex fixation test was normal. The blood tests did not disclose involvement of other organs.

Histopathological findings in the skin

The changes seen in the seven biopsies are summarized in Table 2 and some features illustrated in Figs 4–5. Dermal perivascular and perifollicular lymphocytic infiltration, though minor in one biopsy (case 3), and telangiectasia were found in all biopsies. Quite considerable hyperkeratosis associated with acanthosis, parakeratotic plugging and fibrosis of the papillary layer were found in four biopsies, including one biopsy from each of the three cases.

Immunopathologically (case 1) minimal granular deposits of IgM and more marked deposits of complement C_3 were found at the basement membrane area of affected skin. In the upper part of the dermis considerable deposits of fibrinogen were found. In case 2 no deposits of immunoglobulins, complement C_3 or fibrinogen were found in a biopsy from affected skin of the hand.

Oral findings

Seven of the nine carriers gave a history of recurrent ulceration of the oral mucosa (Table 1). The number of recurrences varied from once a year to once a fortnight and varied in individuals as well. The duration of the ulcers ranged from 4–14 days with an average of 7 days.

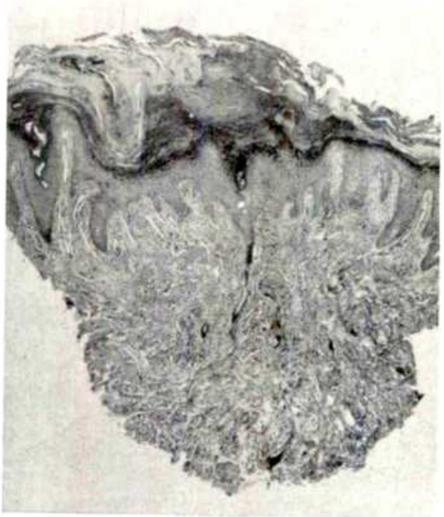

FIGURE 4. Hyperparakeratosis with plugging, irregular acanthosis, telangiectasia of papillary layer, slight lymphocytic infiltration (HE × 32).

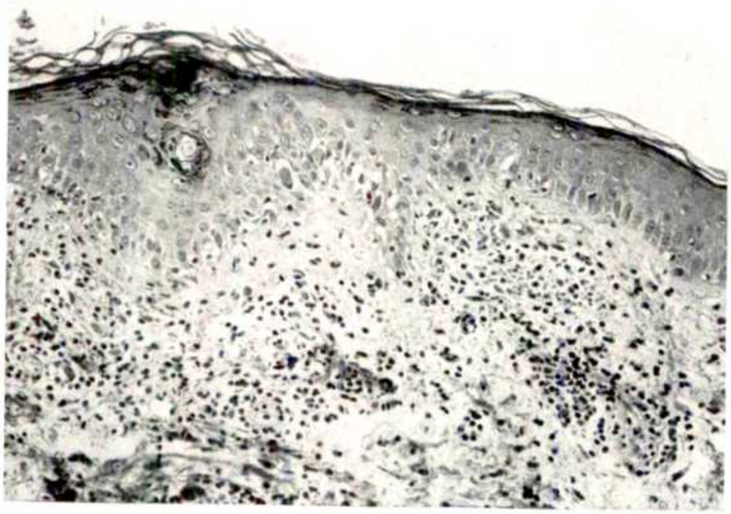

FIGURE 5. Liquefaction degeneration of basal layer with Civatte bodies (HE × 225).

Clinically the five cases (cases 1–5) examined during the active stage showed aphthous-like ulcers located on buccal and labial mucosa (5), gingiva (5), tongue (3), floor of mouth (1) and palate (1) (Figs 6–7). The ulcers had a size of 1–20 mm with the smaller ones dominating (Fig. 6) and were surrounded by a zone of erythema with a width ranging from 0–10 mm. In three cases the ulcers were associated with white areas which were most prominent during the healing stage (Fig. 7). The white changes faded in the course of a few weeks, but were permanent in some areas in one case.

Histopathologically ulceration of the epithelium was found and the connective tissue was infiltrated

TABLE 2. Histopathological findings in affected skin of female carriers of CGD

Case no.	1			2	3		
Biopsy no.	1	2A	2B		1A	1B	2
Scale crust					+		
Plugs	+		+	+			+
Hyperkeratosis	+		+	+	+		+
Acanthosis	+		+	+	+		
Epidermal atrophy		+		+	+		
Liquefaction degeneration of basal layer		+	+	+	+		
Thickened basal membrane				+			
Epidermal lymphocytes	+	+	+	+	+		
Epidermal lymphocytes and vesicles				+	+		
Dermal lymphocytes	+	+	+	+	+	+	+
Subcutaneous lymphocytes	NS	+	+	NS	NS	NS	NS
Oedema of papillary layer		+	+	+		+	+
Fibrosis of papillary layer	+	+	+	+			+
Telangiectasia	+	+	+	+	+	+	+

NS: No subcutaneous tissue in the biopsy.

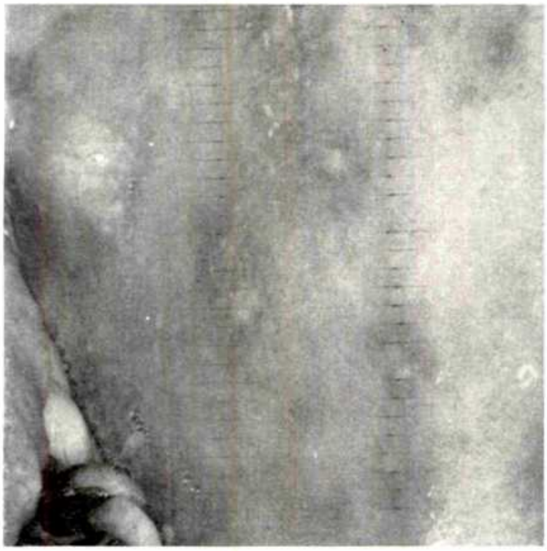

FIGURE 6. Case 3. Multiple small ulcers on left buccal mucosa in carrier for CGD. Note similarity to recurrent aphthous stomatitis of herpetiform type.

by inflammatory cells, predominantly lymphocytes. Corresponding to the white areas mentioned previously the epithelium showed slight to moderate hyperparakeratosis and acanthosis, and a diffuse inflammatory infiltrate, dominated by lymphocytes, was seen in the connective tissue. Epithelioid cell granulomas were not found.

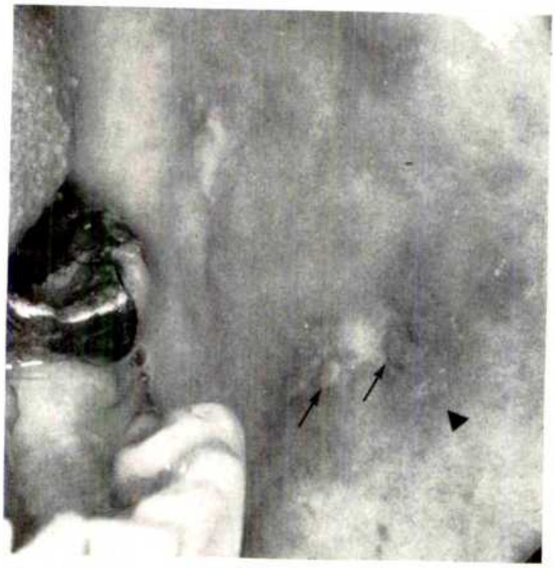

FIGURE 7. Case 5. Ulcers on left buccal mucosa (arrows) in carrier for CGD. Note white area in relation to ulcers and broad zone of erythema (triangle).

Immunopathologically the oral lesions showed deposits of fibrinogen at the basement membrane area in all four cases examined and granular deposits of complement C_3 in two cases. Deposits of immunoglobulins were not demonstrated. IF staining of clinically normal oral mucosa was negative.

DISCUSSION

Most previous reports contain only brief reference to skin diseases in female carriers of CGD, but a few more detailed case reports have been published (Schaller, 1972; Humbert *et al.*, 1976; Nelson, Dal & Goltz, 1977). Three cases (Schaller, 1972, Humbert *et al.*, 1976) had LE-like lesions predominantly of the hands and the face, like our patients. One carrier (Schaller, 1972) also had Raynaud's phenomenon, episodes of arthralgia, pleurisy, stomatitis, and unexplained fever, but a diagnosis of systemic LE was not definitely established. Our series was unselected and showed a remarkably high frequency of skin symptoms.

The photosensitivity, though decreasing with age, the cutaneous manifestations and their response to antimalarials in our patients are in many respects consistent with DLE. The skin changes on fingers and toes are similar to chilblain LE (Millard & Rowell, 1978). The deeper rosacea-like infiltrates on the face and the sterile, subcutaneous abscesses have features in common with LE profundus (Eickstedt & Hassenpflug, 1962). Histopathologically the findings ranged from almost pure lymphocytic infiltration of the Jessner type to lesions consistent with LE, and biopsies with changes similar to chronic dermatitis. Since these lesions are clinically of the same type and do occur with a variable histology in the same patient we find it reasonable to interpret our findings as a pleomorphic type of DLE. The slight lymphocytic infiltration between fat cells in the two biopsies in which subcutaneous tissue was present and hypertrophic changes in other biopsies are consistent with LE-hypertrophicus et profundus (Bechet, 1942; Eickstedt & Hasselpflug, 1962; Dammert, 1971; Otani, 1977). Pigmented histiocytes of the type described by Symchych, Wanstrup & Andersen (1968) in children with CGD were not found.

The stomatitis reported previously in CGD carriers has been interpreted as recurrent aphthous stomatitis (RAS) (Windhorst *et al.*, 1968; Douglas *et al.*, 1969). The recurrent ulcerative stomatitis in our study is comparable with RAS with regard to time of onset, number of recurrences and duration of attacks. Furthermore the ulcers bear an apparent similarity to RAS. However, differences exist: 1. The erythematous halo is of varying width and more extensive than usually seen in RAS. 2. White areas are seen in relation to the ulcers, which histologically show hyperparakeratosis. 3. The ulcers occurred on the attached gingiva in all cases, whereas RAS only rarely affects the gingiva. 4. The IF examination did not reveal deposits of immunoglobulins in contrast to what is found in RAS (Donatsky & Dabelsteen, 1977). It seems, therefore, justified to distinguish between the recurrent stomatitis seen in CGD carriers and RAS.

One of our patients had acute glomerulonephritis in childhood and another patient sequelae after chorioretinitis. Other diseases with an immunological pathogenesis have been reported in carriers of CGD—rheumatic fever and rheumatic heart diseases (Macfarlane, Speirs & Sommerville, 1967; Windhorst *et al.*, 1968), ulcerative colitis (Windhorst *et al.*, 1968) and polyarthritis (Thompson & Soothill, 1970; Schaller, 1972).

Humbert *et al.* (1976) investigated nineteen female LE patients (11 DLE, 8 SLE) for the presence of laboratory findings characteristic of the carrier state for CGD, but none of these patients turned out to have this abnormality. In future screening for this defect among female LE patients it might be beneficial to select a subgroup with recurrent stomatitis, as this symptom seems to be common in the carrier state as shown in our study.

LE has not been reported in patients with CGD. This may be because of the rarity of LE in males or because most boys with CGD have not yet reached adulthood. Since the prognosis is steadily improving on correct diagnosis and treatment it should be important to look for similar skin and mucous membrane symptoms in boys with CGD.

As shown in our study the clinical manifestations in CGD carriers vary considerably in severity and extent. Evidence of uneven distribution of abnormal to normal PMN in carriers has been presented (Biggar, Buron & Holmes, 1976; Repine *et al.*, 1975), and Thompson & Soothill (1970) suggested a correlation between the individual capacity for NBT reduction and the presence of LE-like symptoms. Also, varied involvement of the monocyte-macrophage cell line may be inferred as a pathogenetic factor (Rodey *et al.*, 1969; Lehrer, 1975).

There is no straightforward explanation for the remarkably high incidence of skin symptoms in carriers of X-linked CGD. The stomatitis might result from impaired local defence against oral microorganisms. Stomatitis is thus a frequent finding in affected homozygous boys (Johnston & Newman, 1977). The characteristic skin symptoms are, however, not common in CGD. It may be speculated that a defect in the 'scavenger function' of phagocytes might lead to delayed, or inappropriate elimination of infectious agents, damaged cells and tissue components. This could lead to persistence of antigenic material or to delayed inactivation of biologically active factors. The presence of LE-like lesions in heterozygotes and the absence of these signs in homozygotes might thus be related to the presence in heterozygotes, of both defective and normal phagocytes, the latter being able to respond normally to an abnormal inflammatory stimulus.

The diagnosis of the carrier state of X-linked CGD is of utmost importance for genetic counselling before pregnancy. Not only is it possible to determine the sex of the fetus by amniocentesis, but it is also possible to diagnose CGD prenatally in a male fetus using blood obtained by fetoscope from placental vessels (Newburger *et al.*, 1979). Quite recently (Fikrig *et al.*, 1980) decreased capacity for NBT reduction of skin fibroblasts from CGD patients and carriers, compared to normal skin fibroblasts and cultured amniotic fibroblasts, has been reported. This offers a possibility of prenatal diagnosis of CGD without the hazards of puncture of placental vessels. The novel finding of reduced

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capacity for NBT reduction in skin fibroblasts may also be pertinent to the skin symptoms in carriers of CGD.

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Loss of β_2 microglobulin from the cell surface of cutaneous malignant and premalignant lesions

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SUMMARY

Biopsies from twenty-three patients with malignant skin lesions and from twenty-two patients with premalignant or benign skin lesions were stained for β_2 microglobulin by use of the immunoperoxidase technique. The results showed a significant loss of demonstrable surface β_2 microglobulin from the surface of malignant cells compared to benign, and a partial loss in the premalignant cases. This difference could prove to be a useful tool in the histological diagnosis of malignant skin lesions, and in assessing whether or not the lesion has been completely excised.

Human histocompatibility antigens (HL-A) have been shown to consist of two polypeptide chains—one heavy chain, molecular weight 30,000, which carries the antigenic specificity of the molecule, and one light constant chain, molecular weight 12,000 (Cresswell, Turner & Strominger, 1973). β_2 microglobulin, found in the urine of patients with renal tubular failure and in high concentration on the surface of human leucocytes, has been reported to be biochemically identical to the light constant chain of HL-A (Peterson, Rask & Lindblom, 1974). It has also been demonstrated, by immunological capping experiments, to be closely associated structurally or functionally to HL-A antigens on the cell membrane (Solheim & Thorsby, 1974). Studies of the distribution of β_2 microglobulin have shown it to be present on the cell surface of most normal human nucleated cells so far investigated (Governa & Biguzzi, 1976), including cultured cell lines and lymphocytes, with the exception of Daudi cells (Nilsson, Evrin & Welsh, 1974).

The results of an immunofluorescence survey of the binding of anti β_2 microglobulin to epidermal skin sections indicated that, as expected, β_2 microglobulin is consistently found on the surface of normal human epidermal cells (Forsum & Tjernlund, 1977). A study of basal cell carcinomas, also using immunofluorescent techniques, failed to demonstrate β_2 microglobulin on the membrane of malignant cells in fifteen out of twenty cases (Tjernlund & Forsum, 1977). This suggests a possible identifiable change in the cell surface of neoplastic cells with regard to HL-A expression compared to normal cells, which may be important for cell recognition and interactions determining the behaviour of these malignant cells.

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In this study we have examined skin biopsies from various skin malignancies as well as premalignant and benign conditions to investigate further the possible loss of expression of β_2 microglobulin from the surface of malignant cells. The material has been examined by use of the immunoperoxidase technique, which enables antigens to be demonstrated in stored fixed tissues.

MATERIALS AND METHODS

Skin samples

Formalin fixed, paraffin embedded skin biopsies from patients with histologically diagnosed kerato-acanthoma, basal cell papilloma, actinic keratosis, squamous cell carcinoma, basal cell carcinoma, Bowen's disease and primary cutaneous malignant melanoma were obtained from the routine files of the Department of Dermatology, Western Infirmary, and also from the Department of Pathology, Glasgow Royal Infirmary (kindly obtained for us by Dr F.M.McGregor).

Paraffin sections were cut at 6μ and stored until required.

Immunoperoxidase technique

The immunoperoxidase method used was adapted from that of Taylor and Burns (1974). Slides were immersed in methanol with 0·2% HCl and 0·1% H_2O_2 to remove endogenous peroxidase, then incubated with 1/5 dilution (in 0·01 mol/l Tris HCl pH 7·6) of normal swine serum to block non-

TABLE 1. Details of patients studied with malignant skin lesions

Bowen's disease	Age	Sex	Body site
Case 1	76	F	Leg
2	72	F	Leg
3	82	F	Hand
4	73	M	Hand
5	75	F	Foot
6	72	F	Leg
Squamous carcinoma			
7	78	M	Lip
8	66	M	Cheek
9	40	F	Lip
10	65	M	Lip
11	83	M	Cheek
12	54	F	Cheek
Basal cell carcinoma			
13	57	F	Lip
14	44	F	Lip
15	42	F	Lip
16	67	F	Eyelid
17	63	F	Nose
18	60	M	Face
Primary malignant melanoma			
19	75	F	Calf
20	41	M	Shoulder
21	60	M	Thigh
22	54	M	Face
23	78	F	Arm

TABLE 2. Details of patients studied with premalignant and benign skin lesions

	Age	Sex	Body site
Actinic keratosis			
Case 1	70	M	Temple
2	67	M	Temple
3	58	F	Temple
4	62	M	Hand
5	75	F	Leg
6	66	F	Nose
7	73	M	Nose
8	71	F	Cheek
9	68	M	Cheek
10	58	M	Cheek
Basal cell papilloma			
11	62	M	Thigh
12	60	M	Face
13	56	F	Breast
14	81	M	Thigh
15	46	F	Breast
16	53	F	Thigh
Keratoacanthoma			
17	60	F	Eyebrow
18	65	F	Nose
19	40	M	Hand
20	51	F	Arm
21	80	M	Hand
22	89	F	Nose

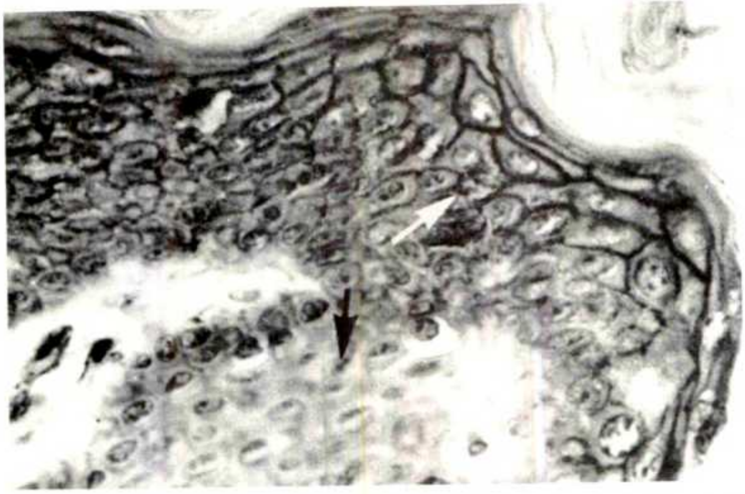

FIGURE 1. Normal skin adjacent to squamous carcinoma. Note the clear deposits of β_2 microglobulin between the adjacent normal epidermal cells (white arrow) and the lack of staining between the cells of the lesion (black arrow).

specific staining. The slides were then sequentially incubated with 1/50 dilution of rabbit anti β_2 microglobulin, 1/100 dilution of swine anti-rabbit immunoglobulin, and 1/100 dilution of peroxidase anti-peroxidase complex (PAP), all obtained from Dakopatts A/S Copenhagen (U.K. supplier—Mercia Diagnostics) with washes in Tris saline (nine parts 0·85% NaCl: one part 0·01 mol/l Tris HCl pH 7·6). Following incubation with 0·03% 3 3′ diamino benzidinete trahydrochloride (Sigma) with 0·02% H_2O_2, which reacts with the bound peroxidase enzyme to form a dark brown product, slides were lightly counterstained with haematoxylin and mounted on glass coverslips with piccolyte resin.

RESULTS

Skin biopsies from forty-five patients with benign, premalignant or malignant skin conditions were stained for β_2 microglobulin as described (Tables 1 and 2). A characteristic pattern of strongly

TABLE 3. Results of immunoperoxidase staining of skin biopsies for β_2 microglobulin

Diagnosis	No. of cases	Positive staining on normal epidermis	Positive staining in tumour/abnormal area	Faint patchy staining in tumour/abnormal area	Negative in tumour/abnormal area
Benign					
Keratoacanthoma	6	6	5		1
Basal cell papilloma	6	6	5	1	
Premalignant					
Actinic keratosis	10	10	2	7	1
Malignant					
Squamous cell carcinoma	6	6		3	3
Bowen's disease	6	6	1		5
Melanoma	5	5			5
Basal cell carcinoma	6	6			6

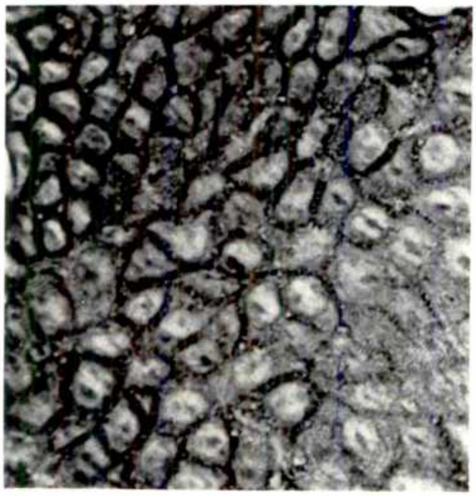

FIGURE 2. Basal cell papilloma. Note clear positive intercellular staining.

positive brown interepithelial staining (Fig. 1) for β_2 microglobulin was seen in the normal epidermis adjacent to the lesional skin of almost all cases (Table 3).

In the benign conditions studied, six cases of keratoacanthoma and six of basal cell papilloma, strong intercellular staining was observed both in normal and in abnormal areas (Fig. 2) of ten out of twelve cases. Of twenty-three cases of skin malignancy, comprising six squamous cell carcinomas, six basal cell carcinomas, six Bowen's disease and five primary malignant melanomas, nineteen showed no intercellular staining (Fig. 3). One case of Bowen's disease showed strongly positive staining on the surface membrane of the tumour cells and three of the squamous cell carcinomas showed some very faint and patchy intercellular staining. This sparse, usually weakly positive, staining of lesional skin was also observed in seven out of ten cases of actinic keratosis, a premalignant condition. Of the remaining three cases, two were strongly positive and one was negative.

Table 4 gives a summary of the results expressed as percentage loss of expression of β_2 microglobulin as detectable by the immunoperoxidase staining.

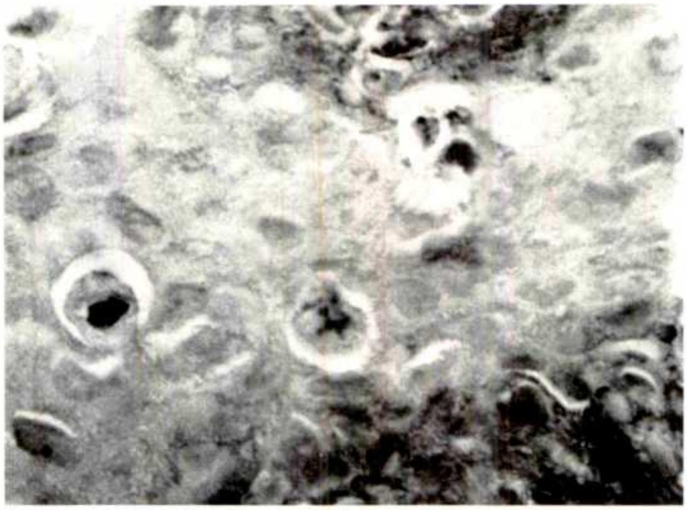

FIGURE 3. Bowen's carcinoma *in situ*. Note lack of intercellular staining.

TABLE 4. Percentage loss of intercellular β_2 microglobulin staining

Diagnosis	% Complete loss β_2	% Partial loss β_2	Total
Benign conditions	8·3 (1/12)	8·3 (1/12)	16·6
Premalignant conditions	10·0 (1/10)	70·0 (7/10)	80·0
Malignant conditions	82·7 (19/23)	13·1 (3/23)	95·8

DISCUSSION

This survey has shown that there is a significant loss of β_2 microglobulin demonstrable by the immunoperoxidase technique on the surface of epithelial cells in primary malignant skin tumours compared to benign proliferative skin conditions, and that a partial loss may be seen in premalignant conditions. This upholds the hypothesis that expression of β_2 microblobulin is lost from the surface of malignant skin cells.

These findings are in agreement with those of Tjernlund and Forsum (1977) who demonstrated loss of expression of β_2 microglobulin from basal cell carcinoma cells by the use of immunofluorescence. We have further shown that this is also true for other skin malignancies: Bowen's disease, cutaneous malignant melanomas, and squamous cell carcinoma.

The use of the immunoperoxidase technique offers significant advantages over the immunofluorescence technique for this type of study. Firstly, a permanent preparation is obtained which can be examined and recorded by conventional microscopic techniques, and secondly, the use of paraffin embedded tissue in this study has enabled β_2 microglobulin staining to be sought retrospectively. This is of considerable importance in the possible application of our observations in the determination of the benign or malignant nature of a cutaneous tumour as sections can be cut from the block used by the histopathologist for conventional stains. In contrast, the use of the immunofluorescence technique requires a decision prior to excision of the tumour and snap freezing of a portion of the tumour after excision, resulting in loss of part of a possibly malignant lesion for normal processing and study. In the field of premalignant and malignant cutaneous lesions loss or distortion by ice artefact of a portion of the lesion is a serious disadvantage. A further point is that our observations may enable accurate demarcation between areas of malignant change and normal tissue to be made. This is of great importance to the pathologist in determining whether or not a malignant lesion has been completely excised, but such a statement can only be made if all the tissue is available for blocking out and orientation.

There are various possible explanations for the failure to detect β_2 microglobulin on the surface of malignant cells.

1. It is possible that the cells are replicating so rapidly that insufficient β_2 microglobulin molecules are being synthesized and are therefore present on the cell surface in quantities undetectable by this technique. In order to test this theory we investigated a case of psoriasis, a non-malignant condition with a well documented high turnover of cells (Weinstein & Frost, 1968), and found characteristic intercellular staining for β_2 microglobulin in the rapidly dividing basal layer of the epidermis. This finding, in conjunction with the results of staining of the benign basal cell papillomas where strongly positive immunoperoxidase staining was seen in the whole lesion of almost all cases, implies that this is not the most likely explanation of our results.

2. The cell surface of the malignant cells could have been altered in such a way that the β_2 microglobulin molecule is no longer available to specific antibody, i.e. a form of steric hindrance to antigen–antibody binding, or that the peptide chain itself has been altered and is antigenically different enough to no longer react with specific antibody.

3. β_2 microglobulin is no longer synthesized by these cells due to changes either in the genetic material of the cell or in the synthetic pathways and is therefore completely absent from the cell surface.

The apparent partial loss of the β_2 microglobulin molecules from the surface of premalignant cells as seen in the ten cases of actinic keratosis studied could be explained by any of the hypotheses.

Whatever the explanation for the absence of β_2 microglobulin from the cell surface of malignant and premalignant cells, it would appear from present knowledge about the functions of the HL-A system that absence of HL-A molecules results in loss of cell-cell recognition. These tumour cells might therefore become increasingly unresponsive to interaction and control from surrounding cells.

ACKNOWLEDGMENT

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The presence of β_2 microglobulin on the membrane of the keratinocyte in premalignant skin disorders

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SUMMARY

β_2 Microglobulin, the invariable light chain of the histocompatibility antigen, has proved to be absent on the cell surface of basal cell carcinoma.

In actinically damaged skin and in patients with a past history of arsenic ingestion changes can be observed on the epidermal cell membrane which can predict malignancy before cell dysregulation is visible. The epidermis in the basal cell naevus syndrome behaves in this respect as normal epidermis and the spontaneous tumour growth cannot be explained by a predisposing defect in the HLA-antigens of the epidermal cell surface.

Various receptor systems are located on the cell surface. One of these receptors is formed by the histocompatibility system (HLA) and has the responsibility for the recognition and lysis of non-self or altered cells (Mahrle & Orfanos, 1977).

The histocompatibility antigens are immunoglobulin-like structures with a variable heavy chain and an invariable light one. They are found on the surface of all human nucleated cells (Evrin & Nilsson, 1974; Mahrle & Orfanos, 1977; Solheim, Rankin & Holmboe 1975). They can be modified after mitogenesis, viral infection and in chemically induced tumour cells (Tsakraklides *et al.*, 1974). The light chain of this HLA antigen is known as β_2 microglobulin (β_2m) and can be demonstrated by immunofluorescence techniques (Solheim, Rankin & Holmboe, 1975). Tjernlund & Forsum (1977) investigated the presence of β_2 microglobulin on the cells of different types of basal cell carcinoma and found that it was absent on the cell surface of tumour-cells in most of the biopsies examined (Tjernlund & Forsum, 1977). In solid basal cell carcinoma sometimes an irregular distribution of β_2m was found (Tjernlund & Forsum 1977). The purpose of the following study is to detect significant alterations

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in β_2m distribution on the epidermal cells of actinically damaged skin and in patients with a history of arsenic medication, and so predict the occurrence of malignancies.

Secondly we investigated how the epidermis and tumours of patients with basal cell naevus syndrome behave with regard to β_2 microglobulin receptor distribution.

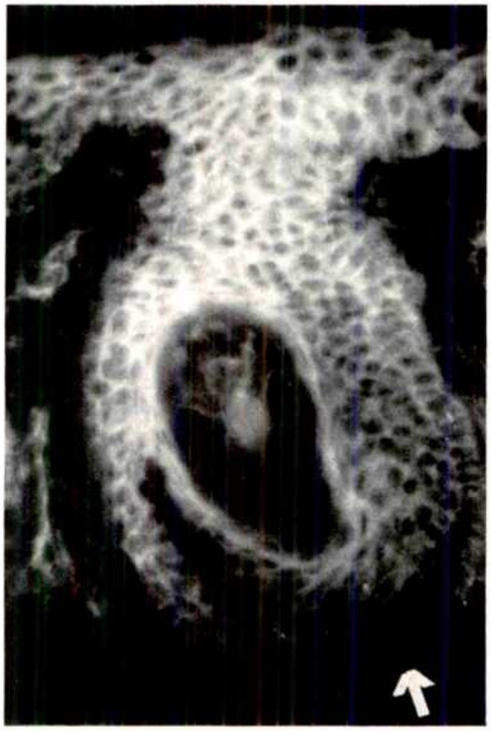

FIGURE 1. Negative fluorescence with anti-β_2 microglobulin in part of hair follicle (arrow) of a patient with actinically damaged skin.

MATERIALS AND METHODS

Patients

Eleven patients with sixteen basal cell carcinomas were involved in the present investigation. The diagnosis was based on clinical and histopathological evaluation.

One of the patients had a basal cell naevus syndrome and another had a hamartomatous basal cell carcinoma on the back.

The histopathological diagnosis in sixteen biopsies was basal cell carcinoma, ten of which showed a solid growth, four were of the sclerosing type with narrow strands of tumour cells embedded in dense fibrous stroma, and two were of a mixed type.

The other eleven biopsies were classified as actinic keratosis (× 5), röntgendermatitis (× 2) and four biopsies were taken from the paralesional skin of patients with multiple malignant lesions (Table 1). As controls there were six biopsies taken from healthy skin of patients who visited our department for other reasons.

TABLE 1. Epidermal location of β_2m in premalignant conditions

Clinical diagnosis	Malignancies in other parts of the skin or predisposing factors	β_2m fluorescence	
		Epidermal	Hair follicle (if present)
1. Actinic keratosis	Multiple, had taken arsenic	−	
2. Röntgendermatitis	None	+	
3. Actinic keratosis	Multiple basal cell ca	−	
4. Actinic keratosis	Multiple basal cell ca	+	
5. Actinic keratosis	None	+	
6. Röntgendermatitis	None	+	
7. Normal skin	Multiple, had taken arsenic	+/−	−
8. Actinic poikiloderma	Multiple basal cell ca	+	
9. Normal skin	Multiple basal cell ca	+	−
10. Normal skin	Basal cell naevus syndrome	+	+
11. Actinic poikiloderma	Multiple basal cell ca	+/−	−

IMMUNOFLUORESCENCE STUDIES

Punch biopsies (4 mm) were taken from the skin under local anaesthesia with chlorethyl spray. The specimens were quick frozen in liquid nitrogen and stored at −70°C.

Cryostat sections were cut at 4 μ and air dried on a microscope slide for 20 min. Next they were washed with PBS (pH 7·2) for 30 min and incubated with the conjugate (anti β_2 microglobulin, Dakopats: dilution 1:40 and 1:80) in a moist chamber. Finally they were washed twice with PBS (pH 7·2). A Leitz Orthoplan microscope with a Xenon XBO-75 lamp and a Leitz vertical illuminator was used. FTC fluorescence was detected using the filter combination BG 39 (4 mm), KP 490 (2 mm) and a secondary filter OG 515 (3 mm). Leitz water immersion objectives (× 50 and × 90) were used.

RESULTS

In fourteen of the sixteen basal cell carcinomas β_2m could not be shown on the wall of the tumour cells.

The hamartomatous variant of basal cell carcinoma and the tumour of the patient with basal cell naevus syndrome behaved similarly to the others.

In two solid growing lesions cell nests with less brilliant fluorescence could be seen while other parts of the tumour were completely negative. Generally the epidermis, lying above, showed a brilliant intercellular fluorescence; twice, however, among this normal pattern we could discern fields with less intense or negative fluorescence. In both cases the clinical diagnosis was actinically damaged skin with recurring basal cell carcinoma.

In the other group with eleven biopsies of actinic keratosis, röntgendermatitis, normal and actinically damaged skin of patients with multiple skin malignancies (Table 1) the results were as follows: In two cases of röntgendermatitis the epidermis showed a normal fluorescence pattern, and so did the 'uninvolved' or non-tumour epidermis in basal cell naevus syndrome.

In the two patients treated with arsenic in the past and bothered now by multiple skin malignancies, the epidermis showed no fluorescence in one case and a less bright to negative fluorescence in the other. One actinic keratosis lesion of a patient with multiple basal cell carcinomas, without a past history of arsenic ingestion, showed a negative epidermal pattern.

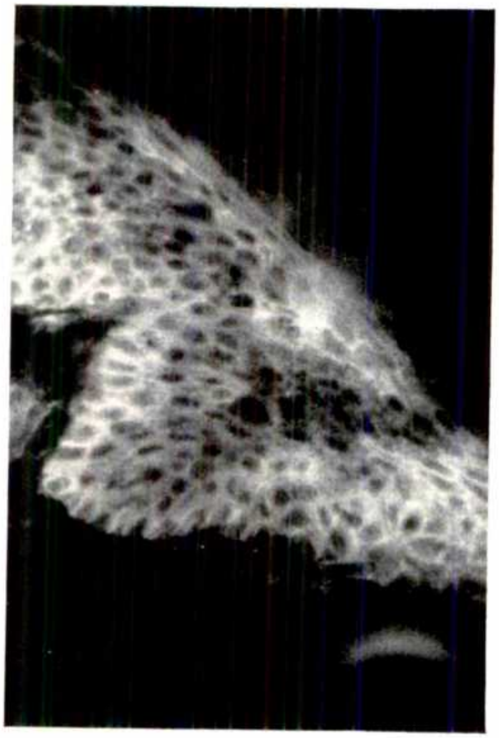

FIGURE 2. Irregular fluorescence with anti-β_2 microglobulin in epidermis of a patient who had taken arsenic in the past.

Twice the normal skin of patients who had been treated regularly for basal cell carcinoma showed a negative fluorescence pattern of the keratinocytes lying next to the hair follicle shaft (Table 1, patients 9 and 11).

DISCUSSION

In normal human skin, as in many non-malignant skin conditions, the epidermis shows a brilliant fluorescence pattern with anti-β_2m. In some cases of actinically damaged skin with a history of multiple malignancies on the sun exposed parts of the body we saw an irregular or negative fluorescence. The same epidermal fluorescence pattern was seen in biopsies from non-tumour skin of patients who had used arsenic in the past. The absence of β_2m on the cell surface of basal cell carcinoma could be an explanation for the intercellular dysregulation and uncontrolled growth of malignant cells by loss of contact inhibition. In accordance with previous investigations of Tjernlund & Forsum (1977) we saw an irregular fluorescence pattern in two cases of solid basal cell carcinoma. The incomplete loss of the membrane marker could be an explanation for the rather non-invasive growth of solid basal cell carcinoma compared with the sclerosing type (Okun & Edelstein, 1976; Andrade *et al.*, 1976). The absence of β_2m on perifollicular cells is strongly suggestive of the adnexal origin of basal cell carcinoma (Pinkus & Mehregan 1976). The disappearance of β_2m from the cell membrane can have different explanations. In the first place the molecule may be modified and not reacting with the specific antibody anymore. A second possibility is that β_2m cannot react with the labelled antibody for some other reason, for instance when it has already formed a complex with circulating anti-β_2m *in vivo*. In the third place the molecule may have disappeared from the cell membrane leaving the receptor site open to other influences. Because of the cell growth regulating potencies of adenyl cyclase the question arises whether there is any relation between β_2m and other receptor systems.

In the case of basal cell naevus syndrome the epidermis shows a normal fluorescence pattern, and the spontaneous tumour growth cannot be explained by a predisposing absence of β_2m on the epidermal cell surface. Summarizing our findings one may assume that such carcinogenic influences as sunlight and arsenic may induce changes on the epidermal cell membrane which can predict malignancy before visible tumour growth has occurred.

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Human dermal microvasculature: II. Enzyme histochemical and cytochemical study

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SUMMARY

An enzyme histochemical and cytochemical study of normal dermal microvasculature showed that respiratory enzymes, lipase and non-specific esterase occurred in all vascular segments. Lysosomal enzymes were also widely distributed and acid phosphatase activity was localized in lysosomes, Golgi apparatus and small portions of endoplasmic reticulum of both endothelial cells and pericytes. Alkaline phosphatase activity, however, was confined to the arterial side and tip of the capillary loop where it occurred in vesicles along the luminal surface of the endothelium and in junctions between endothelial cells. The localization of nucleoside phosphatase activity within the endothelium varied according to substrate; with adenosine triphosphate as substrate, the reaction product occurred in vesicles distributed throughout the endothelial cells; with adenosine diphosphate it was limited to vesicles along the luminal surface; and with adenosine monophosphate, activity was mostly localized to the lateral surfaces of endothelial cells.

These findings suggest functional variation between different vascular segments and between various components of the endothelium.

Attempts to demonstrate a specific Na^+K^+adenosine triphosphatase (transport ATPase) within the endothelium were not successful.

As an extension of our recent light and electron microscopic studies on the segmental organization of normal human dermal microvasculature (Higgins & Eady, 1981) and in an attempt to elucidate further the structure-function relationships of the endothelium and other cellular components of the various vascular segments, we investigated the enzyme histochemical characteristics of this blood vessel network by light microscopy. We also studied the ultrastructural localization of selected enzymes by electron microscopy.

Previous reports concerning enzyme distribution within human dermal blood vessels have been reviewed by Moretti (1968). Most have concentrated upon the alkaline phosphatase reaction in

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different body regions, (Montagna & Ellis, 1957; Kopf, 1957; Ellis, Montagna & Fanger, 1958; Winkelmann *et al.*, 1961; Seifert & Klingmuller, 1972) while others have correlated histochemical with morphological findings in various cutaneous disorders (Nishiyama, 1963 & 1964; Castro & Winkelmann, 1974; Eady & Wilson Jones, 1977). We are unaware of any correlative light and electron microscopic studies on the histochemistry of a wider selection of enzymes in normal human skin.

MATERIALS AND METHODS

Specimens of clinically normal skin from various body regions (including eyebrow, cheek, lip, back, hand and leg) of seven subjects, aged 19–25 years, were processed for light-microscopic histochemistry. Material was collected during the local excision of various lesions as described previously (Higgins & Eady, 1980). Tissue samples were fixed in Baker's formal-calcium, transferred to gum sucrose and frozen sections cut at 10 μm for the detection of alkaline and acid phosphatase (Pearse, 1968), β-glucuronidase, *N*-acetyl-β-glucosaminidase, non-specific esterase and lipase (Pearse, 1972). Fresh frozen material, also sectioned at 10 μm, was used to test for Mg^{++}ATPase, Ca^{++}ATPase, succinate dehydrogenase, lactate dehydrogenase and glucose-6-phosphate dehydrogenase (Pearse, 1968 & 1972).

Further tissue samples, collected in the same manner as outlined above, from thirteen subjects aged from 19–52 years, were used in electron-microscopic cytochemical techniques. After fixation

TABLE 1. Light microscopic enzyme-histochemical results

Test	Ascending arterioles and superficial arteriolar plexus	Terminal arterioles	Capillary loop	Post-capillary venules	Collecting venules and superficial venular plexus
Lactate dehydrogenase	+	+	+	+	+
Succinate dehydrogenase	+	+	+	+	+
Glucose-6-phosphate dehydrogenase	+ +	+ +	+ +	+ +	+ +
Alkaline phosphatase (Burnstone)	—	+ + +	+ + + (*)	—	—
Alkaline phosphatase (Gomori)	—	+ + +	+ + + (*)	—	—
Mg^{++}ATPase	+ + +	+ + +	+ + +	+ + +	+ + +
Ca^{++}ATPase	+ + +	+ + +	+ + +	+ + +	+ + +
Acid phosphatase	±	±	+	+	±
β-glucosaminidase	+	+	+	+	+
β-glucuronidase	+ +	+ +	+ +	+ +	+ +
Lipase	+	+	+	+	+
Non-specific esterase	±	±	±	±	±

* Arterial side and tip of loop only.

— No reaction; ± Equivocal; + Weak reaction; + + Moderate reaction; + + + Strong reaction.

(details of fixation and buffers used in each technique are given in the references below), the material was frozen in the cryostat and sectioned at 40–60 μm. The sections were collected in buffer and transferred to the incubation medium. The fixation and staining methods used to detect activity of the selected enzymes were for alkaline phosphatase (Mayahara *et al.*, 1967); acid phosphatase (Pfeifer, Poehlmann & Witschell, 1975); nucleoside phosphatase activity (White & Krivit, 1965) employing adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and glycerophosphate (GP) as test substrates; Na^+K^+ATPase (transport ATPase) (Ernst 1972a; Russo & Wells, 1977); Mg^{++}ATPase and $Mg^{++}Na^+K^+$ATPase (Russo & Wells, 1977). Control experiments were performed in which substrate was omitted from the incubation media, or where ouabain and parachloromercuribenzoate (PCMB) were used as enzyme inhibitors. In all cases material after treatment was dehydrated in alcohol and embedded in Epon. Ultra-thin sections were cut on a LKB 1 ultramicrotome, collected on uncoated grids, and examined unstained or stained with uranyl acetate followed by lead citrate (Reynolds, 1963) in an AEI EM6B electron microscope.

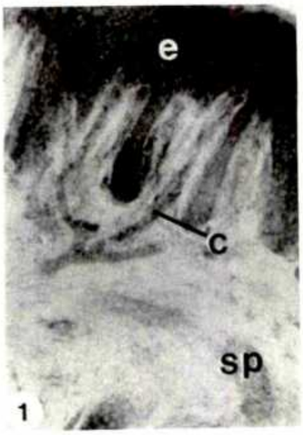
RESULTS

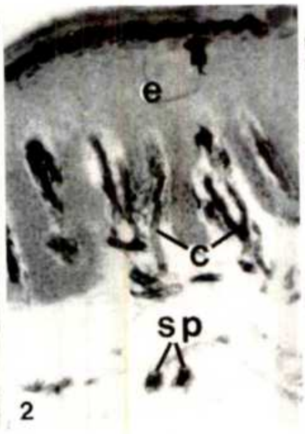
Enzyme histochemistry

The histochemical findings at the light microscope level (Figs 1–3) are summarized in Table 1. No difference in localization or degree of activity of the various enzymes was noted between the different vascular segments, except in the alkaline phosphatase reaction which was limited to the terminal arterioles and arterial side of the capillary loops (for a description of the segmental differentiation of the dermal microvasculature, see Higgins & Eady, 1981).

Enzyme cytochemistry

The ultrastructural localization of alkaline phosphatase activity (Fig. 4) was confined to endothelial cells lining the arterial side of the capillary loop, tip of the loop and that region of the terminal arteriole





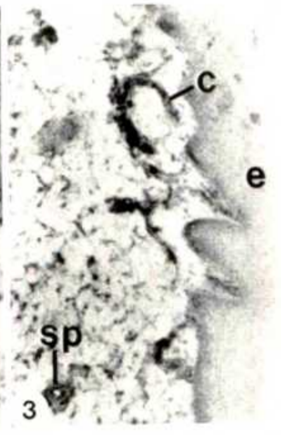
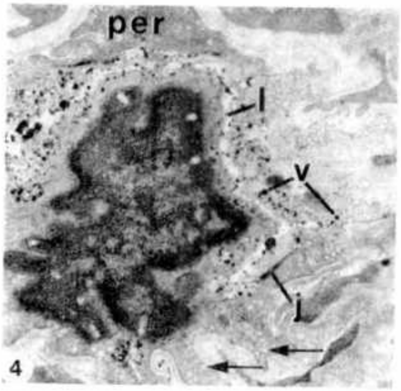
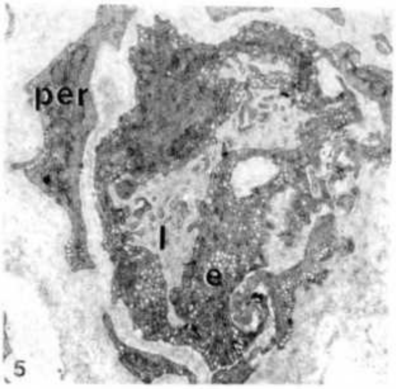


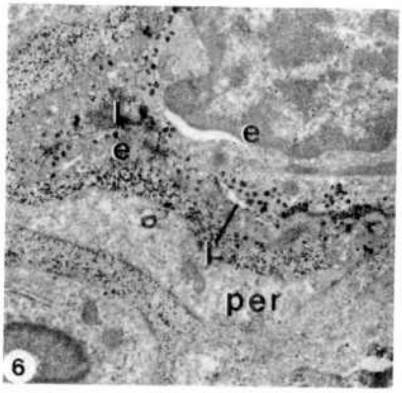
FIGURE 1. Light micrograph showing localization of glucose-6-phosphate dehydrogenase within the capillary loops (c) and deeper vessels of the superificial plexus (sp). e, epidermis (× 130).

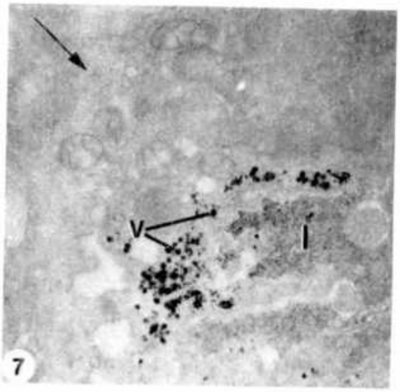
FIGURE 2. Light micrograph showing intense Ca^{++}ATPase activity in both capillary loops (c) and vessels of the superficial plexus (sp); e, epidermis (× 130).

FIGURE 3. Light micrograph showing localization of β-glucuronidase in the microvasculature of human dermis. c, capillary loop; sp, superficial plexus; e, epidermis (× 130).









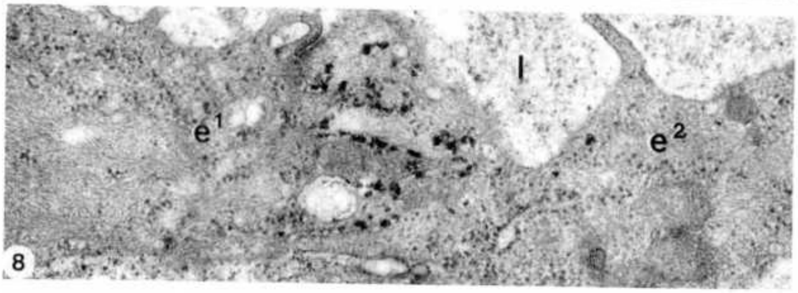


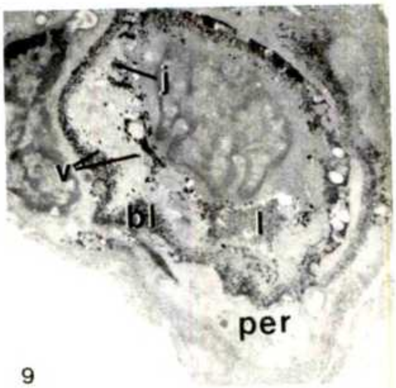
FIGURE 4. Electron micrograph of the arterial side of a capillary loop showing localization of alkaline phosphatase activity. Reaction product can be seen on the luminal surface of the endothelial cells, within vesicles (v) near the luminal surface and on interendothelial cell junctions (j). The vesicles along the abluminal surface of the endothelial cells (arrowed) and in pericytes (per) show no enzyme activity. l, lumen (× 11,000).

FIGURE 5. Electron micrograph of a capillary after incubation for alkaline phosphatase activity in control medium (without substrate). Lead deposit is scarce and shows no pattern of localization. l, lumen; e, endothelial cell; per, pericyte (× 6800).

FIGURE 6. Electron micrograph of part of blood vessel wall demonstrating the localization of ATPase activity. The precipitate is present in vesicles throughout the endothelial cells (e) but not in the pericytes (per). Precipitate (probably non-specific) can also be seen in the lumen (l) and surrounding both endothelial cells and pericytes (× 8200).

FIGURE 7. Electron micrograph showing ADPase activity in the vesicles (v) along the luminal surface of the endothelial cell. The abluminal side of the endothelial cell (arrowed) shows no enzyme activity. l, lumen (× 11,500).

FIGURE 8. Electron micrograph showing localization of AMPase activity. The precipitate is confined to the lateral surfaces of two adjacent endothelial cells (e1 and e2). l, lumen (× 14,400).



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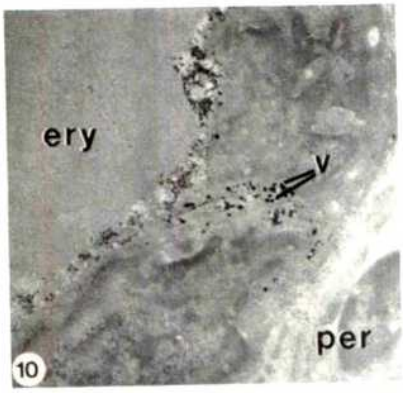

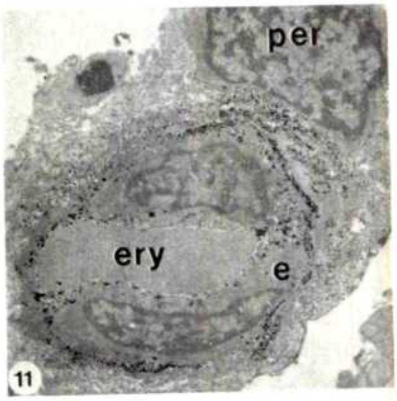

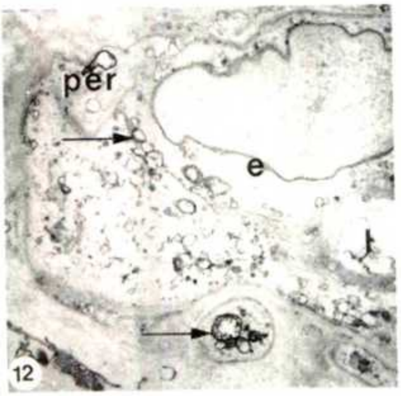

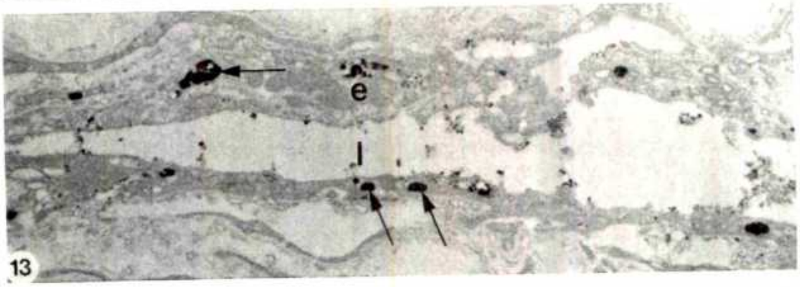

FIGURE 9. Electron micrograph of a transverse section through a vessel in the superficial arteriolar plexus after incubation for Mg^{++} ATPase activity (Russo & Wells, 1977). Lead deposit is present in the vessel lumen (l), endothelial vesicles (v), endothelial cell junctions (j) and basal lamina region (bl). Pericytes (per) show no enzyme activity (× 5800).

FIGURE 10. Part of a blood vessel wall after incubation for $Na^{+}K^{+}$ATPase activity (Russo & Wells, 1977). Precipitate is mostly present along the lateral endothelial cell surface, and the vesicles (v) associated with them. ery, erythrocytes in vessel lumen; per, pericytes (× 10,500).

FIGURE 11. Transverse section through a venule in the mid-dermis after incubation for $Mg^{++}Na^{+}K^{+}$ATPase activity (Russo & Wells, 1977). The distribution of precipitate is similar to that in Fig. 9, for Mg^{++}ATPase. ery, erythrocytes; e, endothelial cell; per, pericyte (× 4600).

FIGURE 12. Electron micrograph of part of capillary loop incubated in medium to demonstrate $Na^{+}K^{+}$ATPase activity, using the method of Ernst (1972 a, b). The non-specific electron dense deposit is localized along the membranes of cell organelles (arrowed) in both endothelial cells (e) and pericytes (per). l, lumen (× 4000).

FIGURE 13. Electron micrograph of longitudinal section through part of capillary loop to show distribution of acid phosphatase activity in lysosomal-like structures (arrowed) within the endothelial cells (e). l, lumen (× 8800).

immediately adjacent to the loop. In these vessels small patches of activity were found on the luminal surface of the endothelial cells. The majority of pinocytotic vesicels near the luminal surface or opening onto the lumen contained reaction product. Junctions between endothelial cells and pinocytotic vesicles in the endothelium close to the junctions were strongly reactive. Alkaline phosphatase activity at the papillary loop tip was mostly concentrated in the endothelium away from the epidermis; the fenestrae were negative. A scattered non-specific deposit was present throughout the lumen. No activity was located in the pericytes. Controls lacking substrate were negative (Fig. 5).

All vessel segments displayed nucleoside phosphatase activity when ATP, AMP and ADP were employed as substrates. The reaction product was localized in vesicles of the endothelium; the degree of staining varying between vesicles in the same cell, and between cells in the same vessel. ATPase activity was widespread in the endothelial cells. Intense activity (Fig. 6) was associated with intercellular junctions and related vesicles. The activity in pericytes was usually absent, but occasionally occurred in a few vesicles and small areas of endoplasmic reticulum. Neither ouabain nor PCMB inhibited this reaction. In comparison, sections incubated in media containing either AMP or ADP as substrate showed a less widespread reaction. ADPase activity (Fig. 7) almost always occurred within vesicles near the luminal side of the endothelial cells, whereas AMPase activity (Fig. 8) was mainly localized to the lateral surfaces of endothelial cells with their associated vesicles: very few vesicles along the luminal and abluminal cell surfaces stained postively. No reaction product was found in tissues incubated with GP as substrate or in the controls lacking in substrate.

Neither the method of Russo & Wells (1977) nor that of Ernst (1972a, b) were considered to be successful in detecting a specific Na^+K^+ATPase. The former technique produced an intense deposit for Mg^{++}ATPase (Fig. 9) and $Mg^{++}Na^+K^+$ATPase (Fig. 11) activity, with a distribution similar to that described above for general ATPase activity. Tissue incubated for Na^+K^+ATPase activity (Russo & Wells, 1977) (Fig. 10) displayed a marked reduction in the reaction product which was mostly limited to the lateral surfaces and associated vesicles of the endothelial cells. In contrast, the method of Ernst (1972a, b, see below) produced no such reaction. The addition of ouabain or PCMB to the incubation medium caused no change in the localization of enzyme activity in any of the test media. Control tissues incubated in media lacking in substrate showed no lead deposit. The method of Ernst (1972a, b) (Fig. 12) produced an electron-dense lead deposit along the nuclear membrane, mitochondrial membranes and the membranes of some pinocytotic vesicles in both endothelial cells and pericytes. The addition of ouabain or the omission of Mg^{++} or K^+ from the medium had no effect on the ultrastructural localization of the lead deposit.

Acid phosphatase activity (Fig. 13) was mostly limited to small bodies of $0{\cdot}20 \pm 0{\cdot}01$ (mean ± s.d.) μm in diameter, probably lysosomes, multivesicular bodies and myelin figures, scattered throughout the cytoplasm of both endothelial cells and pericytes in all vessels examined. Reaction precipitate was also found in the Golgi apparatus and associated vesicles, in small portions of the endoplasmic reticulum, and on the interendothelial cell junctions. No lead precipitate was present in control tissue.

DISCUSSION

The positive histochemical reaction for alkaline phosphatase in the arterial side and tip of the capillary loop, as shown in the present study, has previously been found useful for demonstrating these portions of the microvasculature (Winkelmann *et al.*, 1961; Ellis, 1961; Romanul & Bannister, 1962; Seifert & Klingmuller, 1972). The localization of the enzyme in vesicles associated with the luminal surface of the endothelium may indicate that these vesicles have a different function from those of the rest of the dermal blood vessel network.

Further differentiation amongst endothelial vesicles is suggested by differences in localization of the various nucleoside phosphatases that we tested. ADPase activity was present mainly in vesicles along the luminal surface, AMPase activity in those along the lateral cell surfaces whereas ATPase was widespread in vesicles throughout the endothelial cells. Differences in localization and specificity of neutral phosphatases have previously been demonstrated in rabbit aorta (Hoff & Graf, 1966). The presence of ATPase suggests that these vesicles are involved in an energy requiring process, probably transport, since the smooth-surfaced vesicles associated with the luminal, basal and lateral surfaces of endothelial cells have been shown to play a vital role in transport across the endothelium (Burns & Palade, 1968; Simionescu, Simionescu & Palade, 1973, 1975). A further function attributed to vesicles open to the vascular lumen, and to points of incipient vesicle formation along the plasma membrane, involves hydrolysis by nucleotidases of vasoactive substances as they flow through the vessels, the reaction products being returned to the circulation (Ryan & Smith, 1971 a, b; Leiberman, Westwick & Lewis, 1979). A proportion of the vesicles along the abluminal cell surface may also be involved in basal lamina formation.

The failure to detect Na^+K^+ATPase activity may be due to limitations in the techniques used, the enzyme concentrations being too low to be detected by these methods, or it may indicate a true absence of enzyme activity. The results possibly suggest that the endothelial cells do not have a function in the active transport of water and its solutes across the luminal membrane via a mechanism which involves the efflux of Na^+ coupled directly to the hydrolysis of ATP through Na^+K^+ATPase. If such a mechanism did exist we might expect to find Na^+K^+ATPase along the basolateral membranes of the endothelial cells (Fujita & Nakao, 1973). An ouabain-sensitive Na^+K^+ATPase linked K^+ transport system (Goldstein, 1978) and carrier mediated glucose transport (Goldstein, Csejtey & Diamond, 1977) are, however, well recognized in brain capillaries. Goldstein (1978) found the K^+ uptake by the capillaries of mammary gland was markedly reduced compared to brain capillaries. He suggested that an ion pump is necessary in the brain to maintain the concentration gradient of K^+ across the continuous endothelial cell barrier; the concentrations of ions in systemic capillaries being the same in the plasma due to the gaps between endothelial cells in these vessels. The results do not, however, discount the possibility of some other transport mechanism operating across endothelial cell membranes (Uglem, 1976).

Marchesi (1964) was unable to detect either lysosomes or acid phosphatase activity in the endothelium of vessels not involved in inflammation. Movat & Fernando (1964) noted that lysosomes were abundant in venules but rare in capillaries, arterioles and small arteries. On examining their published micrographs, however, we consider that many of the organelles they assumed to be lysosomes are possibly Weibel–Palade bodies (Weibel & Palade, 1964). The present histochemical study provides evidence for the presence of the lysosomal enzymes β-glucuronidase, β-glucosaminidase and acid phosphatase in normal human dermal microvasculature. The ultrastructural demonstration of acid phosphatase activity in lysosomes, Golgi apparatus with associated vesicles, endoplasmic reticulum, multivesicular bodies and myelin figures supports our earlier suggestion (Higgins & Eady, 1981) that endothelial cells have an endocytotic function.

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Biochemical characterization of tyrosinase inhibitors using tyrosinase binding affinity chromatography

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SUMMARY

Purification of tyrosinase inhibitors of hamster melanomas was carried out using tyrosinase binding affinity column chromatography. This method enables the isolation of tyrosinase inhibitors with a 124-fold purification index as compared to that of crude preparation after dialysation. The purified inhibitors consist of a mixture of 5000–6000 and a 310 molecular weight fraction. They also show characteristics of polypeptides which contain glycine, glutamic acid, serine, proline and alanine as main amino acids.

There have been a number of reports (Flawn & Wilde, 1970; Riley, Hobby & Burk, 1953; Rothman, Krysa & Smiljanic, 1964; Chain & Wilgram, 1967; Cooper & Mishima, 1967; Hamada & Mishima, 1972; Satoh & Mishima, 1967, 1970) describing the naturally occurring tyrosinase inhibitors participating in an intracellular melanogenic control system in pigment cells. These inhibitors can be divided into at least two main groups; one consisting of non-protein, sulph-hydryl compounds which have been found predominantly in various skins, and the other of protein, non-sulph-hydryl compounds from melanoma or liver tissue, both having dopa-autoxidative inhibitory properties. We have previously reported (Cooper & Mishima, 1967; Satoh & Mishmia, 1967, 1970; Hamada & Mishima, 1972) the presence and chemical properties of melanogenic inhibitor(s) in hamster melanoma cells which were heat stable, dialysable and competitive tyrosinase inhibitors. In the present study, in order to obtain further understanding of this inhibitor, additional purification and characterization was carried out using tyrosinase binding affinity column chromatography.

MATERIAL AND METHODS

Materials

Greene's melanotic and amelanotic melanomas originally obtained from H.S.N.Greene were used in this study.

Preparation of crude inhibitor from tissue

Non-necrotic melanoma, excluding epidermis, was homogenized in ice-cold 0·25 mol/l sucrose

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solution using an Ultra Turrax homogenizer and further treated with a glass homogenizer chilled with ice. The suspension obtained was first centrifuged at 700 *g* for 10 min to remove unruptured cells and nuclear debris. The residue was discarded. The supernatant was further centrifuged at 12,000 *g* for 10 min to obtain a soluble fraction. The soluble fraction was treated in a dialysing apparatus using *Hollow Fiber* or *Mini Module* to obtain three different molecular weight (>30,000, 6000–30,000 and <6000) subfractions. The <6000 molecular weight subfraction which had the highest inhibitory activity (Imokawa & Mishima, 1980) was used as a crude inhibitor (CRI) to study its chemical properties and as the starting material for the subsequent purification.

Preparation of tyrosinase affinity column

All procedures were performed using para amino benzyl (PAB) cellulose according to the method of Campbell, Leuscher & Lerman (1951). Thus, 1·6 *g* of PAB cellulose was suspended in 16 ml of 1N HCl solution and reacted with 0·96 ml of $NaNO_2$ (0·5 mol/l) for 15 min by stirring at 40°C until completion of the diazo reaction. The termination of the diazo reaction was checked with KI starch paper. Following a further 15 min stirring, the cellulose was washed with cold 0·01 N HCl and subsequently with cold water through a glass filter until the washes acquired neutral pH. Subsequently, the washed cellulose was suspended in mushroom tyrosinase solution including 50 ml of 0·05 mol/l phosphate buffer (pH 8·0). The coupling reaction was carried out at 40°C for 37–90 h. The cellulose was washed with coupling buffer through a glass filter, and further by the elution buffer (phosphate buffer pH 6·8, 0·05–0·01 mol/l). The amounts of tyrosinase bound to PAB cellulose were estimated from decreased amounts of tyrosinase protein compared to before the coupling reaction.

Affinity chromatography

The fraction with molecular weight <6000 which was obtained after dialyzation (Imokawa & Mishima, 1980) was applied to the column prepared as described. The column measured 3 × 1·5 cm. The material was recirculated three times through the column. After recirculation, the column was washed with 10 or 50 mmol phosphate buffer (pH 6·8) solution until the eluted solution had no absorbance at 260 or 280 nm. Elution of inhibitor from the column was carried out by using discontinuous salt solution or acid pH solution and fractions of 3 ml were collected. Fractions belonging to each peak were combined and concentrated by freeze-drying. An appropriate volume of distilled water was added to the concentrated inhibitor powder for the inhibitor assay. As a control preparation, an equivalent volume of eluted buffer was treated in the same manner as described above to adjust the salt concentration to the same level as in the inhibitor material.

Column chromatography

1·0 ml of the inhibitor solutions was placed on Bio-Gel P-2 or P-3 column (2 × 24 cm) equilibrated with a 0·05 mol/l phosphate buffer (pH 6·8). Elution was performed with the same buffer at the elution rate of 6 ml/5 min. Fractions of 3 ml were collected and monitored at absorbance of 260 or 280 nm.

Inhibitory assay

0·2 ml of an aqueous solution of mushroom tyrosinase (138 unit) was mixed with 0·25 ml of inhibitor solution and 1·05 ml of 0·1 mol/l phosphate buffer (pH 6·8) and then incubated for 1 min at 37°C, after which 1·5 ml of 2 mmol dopa kept at 37°C was added to the mixture to measure immediately the initial rate of linear increase in optical density at 475 nm, based on the formation of dopachrome.

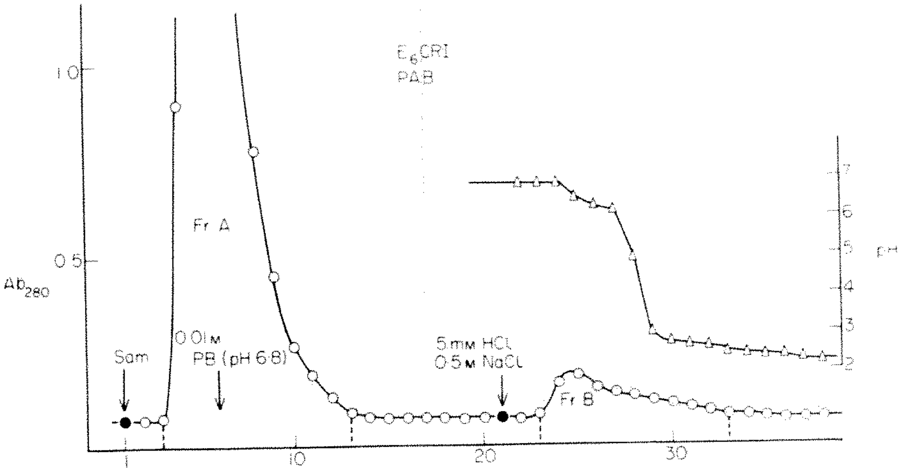

FIGURE 1. Affinity chromatographic pattern of amelanotic tyrosinase crude inhibitor using tyrosinase binding PAB-cellulose column.

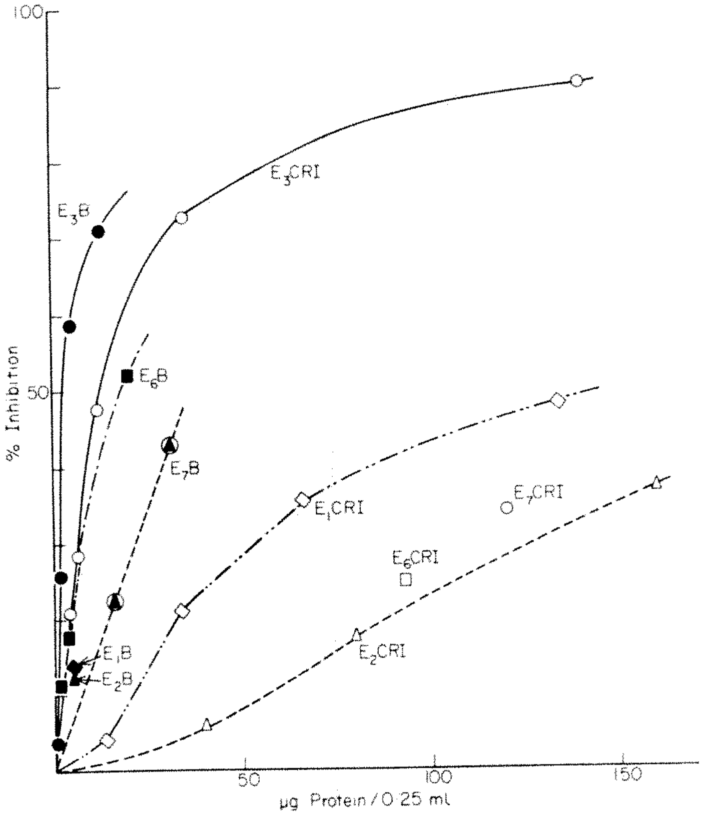

FIGURE 2. Inhibitory activities of tyrosinase inhibitors purified by affinity chromatography from crude inhibitors expressed as a function of the amounts of protein.

TABLE 1. Summary of the results of the main experiments (E_1–E_7) for purification of tyrosinase inhibitors using affinity chromatography. CRI: crude inhibitor preparation, PB: phosphate buffer

Experiment(E) no.	Bound tyrosinase (mg protein)	Cellulose column (g)	Equilibrated buffer (mmol P.B.)	Elution agent	Inhibitory activity (IN$_{50}$%, μg protein/0·25 ml) CRI	Fr. A	Fr. B	Purification ratio	Yield (%)
E_1 Melanotic	1·94	1·0	50	0·1 mol/l Glycine-HCl (pH 2·8)	217	—	22.7	9·6	—
E_2 Melanotic	1·94	1·6	50	0·1 mol/l Glycine-HCl (pH 2·8)	153	—	20·2	7·6	
E_3 Melanotic	4·34	1·6	50	2·5 mmol HCl (pH 2·8)	12·5	65·0	3·75	3·3	11·8
E_4 Melanotic	1·75	1·6	10	5 mmolHCl+ 0·5 mol/l NaCl (pH 2·2)	905	—	—	—	—
E_5 Melanotic	0	1·6	10	5 mmol HCl+ 0·5 mol/l NaCl (pH 2·2)	800		0	—	—
E_6 Amelanotic	2·82	1·6	10	5 mmol HCl+ 0·5 mol/l NaCl (pH 2·2)	2300	5170	18·5	124·3	100
E_7 Amelanotic	6·53	1·6	10	5 mmol HCl (pH 2·0)	2080	5600	37·0	56.2	17.8

The extent of inhibition by the addition of samples was expressed as μg protein concentration necessary for 50% inhibition.

RESULTS

Affinity chromatography

The typical elution pattern (Fig. 1) after fractionation on tyrosinase binding PAB cellulose column results in the emergence of two peaks, the first (Fr.A) emerging as non-binding substance and the second (Fr.B) having inhibitory activity which is eluted first after application of a solution with decreased pH or increased salt concentration of NaCl. The active fractions are combined, concentrated and assayed for inhibitory effect on mushroom tyrosinase which is then plotted against μg protein concentration in 0·25 ml (Fig. 2) and is finally expressed as μg protein required for 50% inhibition. The results on purification of tyrosinase inhibitor using affinity chromatography are summarized in Table 1. It is found that efficiency for purification of tyrosinase inhibitor depends upon the amounts of bound tyrosinase in the column, the kind of equilibration buffer used, and the elutant. Using melanotic crude inhibitor preparation, the inhibitory capacity of the peak increases in proportion to increasing inhibitor activity of crude inhibitor preparation used, while the result of the amelanotic inhibitor preparation shows relatively high efficiency as compared to that of melanotic inhibitor. Further, application of a combined solution of HCl and NaCl as elutant gives better results than that of HCl alone.

Characterization of purified inhibitor

Analysis of amino acids of the purified inhibitor which has been found to show the highest inhibitory activity ($IN_{50\%}$ = 3·57 μg) of all the purified inhibitors, shows the presence of Gly, Glu, Ser, Pro and Ala as their main constituents (Fig. 3).

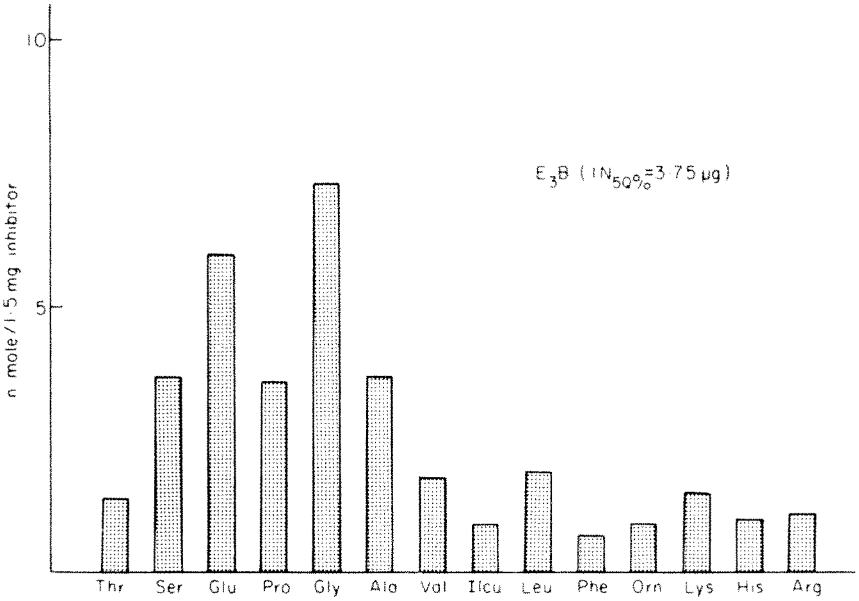

FIGURE 3. Amino acids constituting tyrosinase melanotic inhibitor purified by affinity chromatography.

Further fractionation of the purified inhibitor from melanotic melanoma on Bio-Gel P-2 (Fig. 4) reveals that there is the emergence of (a) and (b) peaks both with inhibitory activity. However, the fractions corresponding to the (a) and (b) peaks which are obtained in this Bio-Gel system of both crude inhibitor and inhibitors obtained after DEAE-Sephadex column chromatography (Imokawa & Mishima, 1980) except for (a) peak from crude inhibitor, are found to have an inhibitory activity. Comparison of affinity chromatography and the DEAE-Sephadex column system in terms of its elution pattern has revealed that, with respect to its molecular distribution, the former technique gives a much higher purification index than the latter. In the case of amelanotic inhibitor, (b) peak but not (a) peak shows inhibitory activity, whereas with crude inhibitor preparation, both (a) and (b) peaks show inhibitory activity.

Dertermination of the molecular weight of (b) peak using Bio-Gel P-2 indicates it to be 310 as shown in Fig. 5. Fractionation of inhibitor purified by affinity chromatography on Bio-Gel P-3 shows (a) peak of Bio-Gel P-2 to be present in the void volume, suggesting that our inhibitor purified by affinity chromatography consists of a mixture of 5000–6000 molecular weight and a 310 one. Based on its inhibitory activity per intensity of absorbance at 260 and 280 nm, the final purification index after Bio-Gel P-2 chromatography in comparison with the crude inhibitor preparation is found to be approximately 400 and 200 times enriched activity respectively for amelanotic, and 90 and 70 times for melanotic melanomas (Table 2).

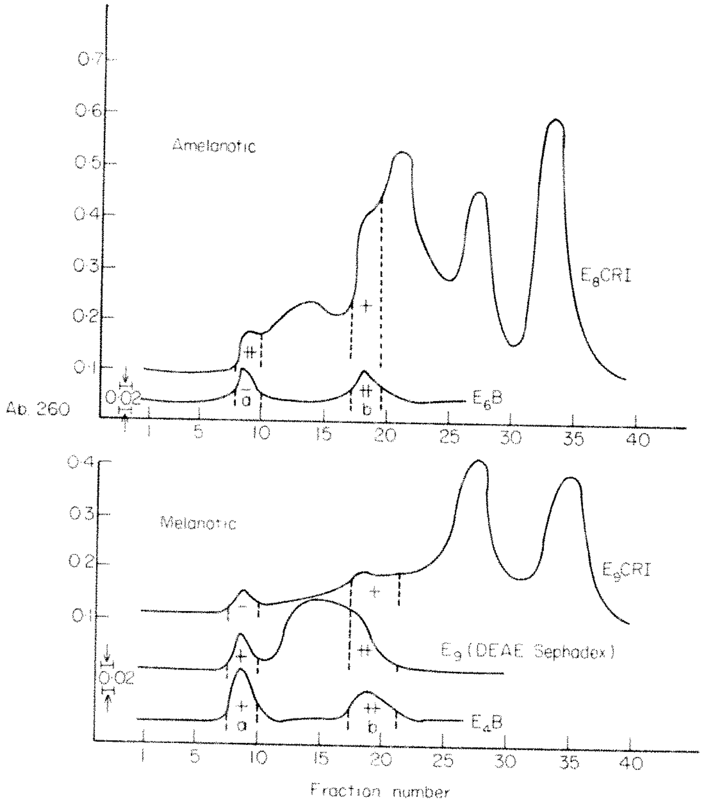

FIGURE 4. Gel chromatographic pattern on Bio-Gel P-2 of tyrosinase inhibitors purified by affinity chromatography in comparison with that by DEAE-sephadex chromatography (Imokawa & Mishima, 1980). —, + and + + show relative inhibitory capacity of the fractions.

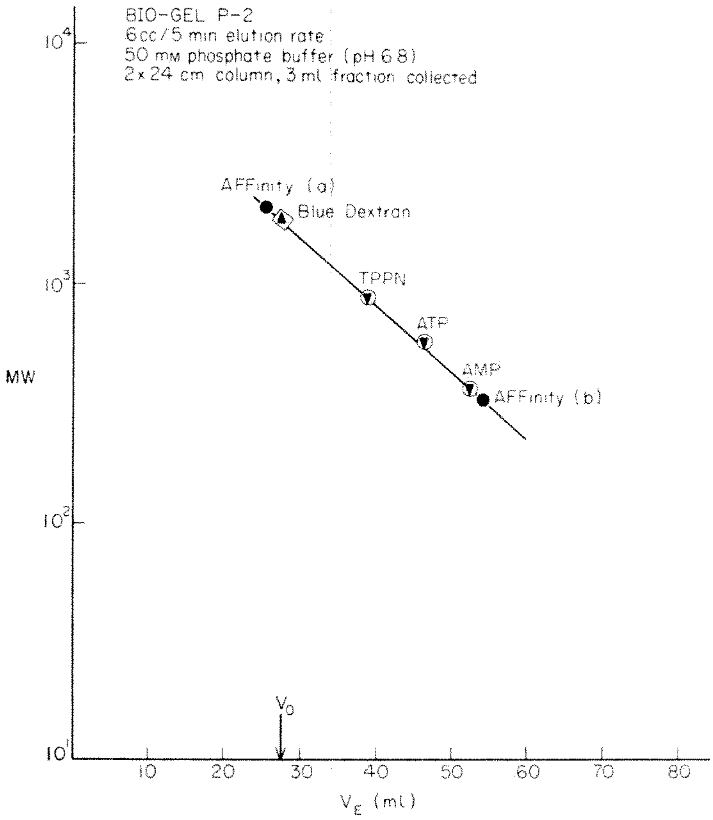

FIGURE 5. Molecular estimation of tyrosinase inhibitor purified by affinity chromatography. TPPN: Triphosphopyrizine nucleotide, ATP: Adenosine triphosphate, AMP: Adenosine monophosphate.

TABLE 2. Results of typical purification procedures shown by inhibitory activities and purification index

		IN $_{50\%}$			Purification index		
		µg protein 0·25 ml	A_{260}	A_{280}	µg protein /0·25 ml	A_{260}	A_{280}
Hollow fiber (MW 6000)	E_6 (Amelanotic)	2300	115·5	67·5	1·0	1·0	1·0
	E_4 (Melanotic)	905	37·6	24·1	1·0	1·0	1·0
Tyrosinase Affinity chromatography	E_6 B	18·5	1·41		124·3		
	E_4 B	—	—	—	—	—	—
Bio-Gel P-2 Gel Chromatography	E_6 B(b)	—	0·44	0·33	—	398·3	204·5
	E_4 B(b)		0·44	0·33	—	85·4	73·0

DISCUSSION

Rothman (1964) described a heat-stable, dialyzable, non-protein sulph-hydryl tyrosinase inhibitor, originating from aqueous extract of human epidermis, as well as its inactivation in sun-tanned skin. Flawn & Wilde (1970) found an inhibitor of dopa autoxidation which was identified as a heat stable, dialyzable and non-sulph-hydryl compound in guinea-pig skin. However, as total skin or epidermal tissue were usually used in their studies, whether these inhibitors truly originate from the melanocyte alone remains unclarified. Since there are descriptions of similar inhibitory materials in tissues which do not contain melanin synthesizing cells, such as liver and blood, their function in such non-pigmentary cells is unknown.

In melanoma cells, which synthesize melanin, the presence of naturally occurring non-sulph-hydryl tyrosinase inhibitors has been reported. Riley, Hobby & Burk (1953) using Cloudman S-91 and Harding-Passey, pointed out an inhibitor of dopa autoxidation which was a heat-unstable and undialysable compound. Cooper & Mishima (1967) postulated, on the basis of a marked inhibitory effect of amelanotic melanoma homogenate on tyrosinase from melanotic melanoma, that the absence of melanization in amelanotic melanoma is related to the presence and concentration of a tyrosinase inhibitor. In addition, it was found that this inhibitor is heat-stable and dialyzable, is not a sulph-hydryl compound and, as judged by the diminished activity after treatment with chymotrypsin and trypsin, is protein in character.

Matsumoto *et al.* (1967) isolated from mouse liver an inhibitor, which is heat-stable and dialysable and of 4000 molecular weight, consisting of a nucleic acid analogue with molecular weight of 700–800 and a polypeptide with a molecular weight of 3000–5000. Our purified inhibitor seems to be similar in its molecular constituents to the liver inhibitor as well as to the inhibitor isolated by Flawn & Wilde (1970) from guinea-pig skin.

In the present study, our method utilizing tyrosinase binding affinity chromatography made it possible to isolate this inhibitor, having a 124-fold purification index as compared to that of the crude preparation after dialyzation. Furthermore, it has been found that this purified inhibitor consists mainly of polypeptides which show Gly, Glu, Ser, Pro and Ala as main amino acids. After subsequent fractionation on Bio-Gel P-2, a fraction with a molecular weight of 320 was shown finally to be 200–400 times richer in inhibitory effect on mushroom tyrosinase as compared to that of the crude inhibitor preparation.

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An action spectrum for 8-MOP induced sunburn cells in mammalian epidermis

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SUMMARY

The histological change known as individual cell shrinkage necrosis (so-called 'sunburn cell' in mammalian epidermis) would seem to be suitable for quantitative morphological studies on the effect of psoralen treatment and near UV irradiation. The number of sunburn cells per unit length of epidermis versus varying doses of narrow waveband UVR, 305–375 nm, has been studied and an action spectrum constructed which had maximal activity at 320–335 nm. The implications of these findings are discussed.

Individual cell shrinkage necrosis is a well known histological phenomenon of distinctive appearance, which is taken to indicate impending cell death (Kerr, 1971). It is a feature of the usual histopathological picture of normal sunburn from UVR (ultraviolet radiation) in the 280–315 nm range, so-called UV-B, in human epidermis (Daniels, Brophy & Lobitz, 1961; Johnson, Mandell & Daniels, 1972), and epidermal cells showing this particular type of change have become known as 'sunburn cells' (SBC). These cells when stained with haematoxylin and eosin show a characteristic pyknotic nucleus and eosinophilic cytoplasm. Near UVR (so-called UV-A, 315–400 nm) does not promote the appearance of sunburn cells (Kumakiri, Hashimoto & Willis, 1977; Willis & Cylus, 1977) unless combined with psoralen treatment (Woodcock & Magnus, 1976). The mechanisms by which psoralens exert their photobiological effects are by no means clear. It is known that these compounds may photoreact with the pyrimidine bases of DNA to produce both 4′, 5′ and 3, 4 mono-adducts and that if both these reactions take place in the same psoralen molecule, a crosslink is possible between opposite strands of DNA (Cole, 1970, 1971; Dall'Acqua *et al.*, 1971). Few studies have been made on the relative biological effectiveness of mono-adducts and crosslinks, but experimental evidence to date suggests that the crosslink reaction has much greater photobiological potency (Dall'Acqua *et al.*, 1974; Coppey, Averbeck & Moreno, 1979; Young, 1980).

Although the significance of the sunburn cell has yet to be established, Woodcock & Magnus (1976) considered DNA to be a possible primary chromophore. Thus the sunburn cell, in mammalian epidermis, may offer a convenient model for certain types of DNA studies and this seems particularly

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important in view of the current widespread use of so-called PUVA therapy for certain dermatological conditions, e.g. psoriasis (Parrish *et al.*, 1974), vitiligo (Kenney, 1971) and mycosis fungoides (Gilchrest *et al.*, 1976).

MATERIALS AND METHODS

Experimental animals

These were male hairless albino mice, 4–6 weeks old; a long established in-bred strain of the Institute of Dermatology, London.

8-MOP

Crystalline 8-MOP (1·85 mM) was dissolved in absolute ethanol. Purity was authenticated by TLC, absorption spectroscopy and mass spectrometry.

Skin psoralen treatment procedure and irradiation

Animals were immobilized in jackets of aluminium foil and double sided sticky tape. Each jacket had a 5 × 5 mm aperture positioned over the mid flank of the mouse. This skin area was then treated with 12·5 μl of psoralen solution or control solvent dispensed by micropipette. An hour and a half after the application of the experimental solution, the animals were irradiated, the skin site being located with a small indian ink tattoo. A double monochromator was used with quartz Littrow prisms and 2 kW xenon arc. Wavelength calibration was made with a medium pressure mercury arc and spectral power was measured with a thermopile. The monochromator had three slits which could be adjusted so that 50% of the energy was in a 12 nm bandwidth, as checked with a recording spectrophotometer. Irradiance ranged from 1·4 mW cm^{-2} in the waveband centred at 305 nm to 4·6 mW cm^{-2} in that at 375 nm.

In investigating a dose-response curve, 4–7 mice treated with 8-MOP, or with control solvent, were used for each dose point. To avoid possible circadian influence, irradiations were always performed at similar times of the day.

Histology and counting of sunburn cells

The animals were sacrificed 20 h after irradiation. The irradiated site was immediately excised, stretched on a × 5 mm card and fixed in Bouin's solution for 24 h. Paraffin sections (8 μm) were prepared. Every 10th section was taken until each of three slides bore 7–8 sections. Staining was with haematoxylin and eosin. Sunburn cell counting was done at a magnification of ×400 and without knowing the treatment that specimens had received. At least twenty different fields of view (0·5 mm) from each slide were examined. Areas in the immediate vicinity of hair follicles were avoided. The mean counts of three slides from each sample were expressed as the number of SBC cm^{-1}.

Statistical treatment of results

Analysis of the sunburn cell counts in the dose response data indicated a log normal rather than a normal distribution. Linear regression analysis was performed with a Genstat 4·01 (1977) package program. Each individual sunburn cell count in a sample was used rather than the mean count at each dose-point. This not only eliminated the need to weight means to offset variation in standard deviation but also allowed full residual analysis and analysis of variance to check the validity of the model $y = \alpha x + \beta$, where $y = \log_{10}$ number of SBC cm^{-1} and $x = \log_{10}$ dose in mJ cm^{-2}.

For every 8-MOP treated area of mouse, there was a matched control on a separate mouse treated with solvent only, *viz.* ethanol. In the UV-B experiments, control treatment did result in sunburn cell production, so 8-MOP and control dose response data were analysed separately. In the UV-A experiments, however, control treatment had little or no effect. Control data were therefore subtracted from 8-MOP data which were then designated 'control corrected'.

RESULTS

Examination of residuals confirmed the normal distribution of the log-log plots at all wavelengths. Analysis of variance showed that all the regressions were highly significant ($P < 0{\cdot}005$) and that all variation in sunburn cell counts at specific dose-points within each experiment could be attributed to experimental error rather than 'lack of fit'.

Figures 1 and 2 are UV-B dose-response curves (UVR dose varied, 8-MOP kept constant) and show that, with wavebands centred at 305 and 312·5 nm, sunburn cells were observed in a dose dependent manner whether or not 8-MOP was given. Regression values are given in Table 1. At 305 nm, the 8-MOP and control slopes were not significantly different ($P > 0{\cdot}05$). This was not the case at 312·5 nm, where the control slope was steeper than the 8-MOP slope ($P < 0{\cdot}02$). The dose-response curves performed with narrow waveband UVR centred at 320, 335, 350, 365 and 375 nm resulted in radiation dose-dependent sunburn cell counts only when 8-MOP was given. Irradiation without 8-MOP had virtually no effect. Table 2 gives the regression parameters of these dose-response curves. The slopes of these lines are not significantly different ($P > 0{\cdot}05$) and are summarized in graphic form in Fig. 3. Figure 4 shows an action spectrum constructed from the regression parameters given in Table 2. The end-point, $\log_{10} 61{\cdot}25$ SBC cm^{-1}, i.e. $y = 1{\cdot}79$, was chosen because it is the median of the means of the counts at both the lowest and the highest dose-points of all UV-A wavebands. At 305 nm, the 8-MOP and the control curves are virtually superimposable, possibly

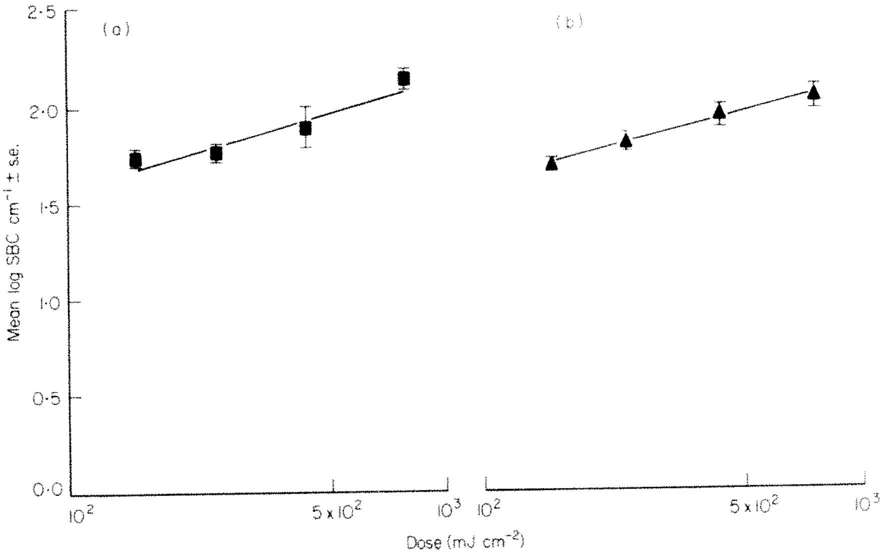

FIGURE 1. (a) 8-MOP dose-response curve at 305 nm. (b) Control dose-response curve at 305 nm.

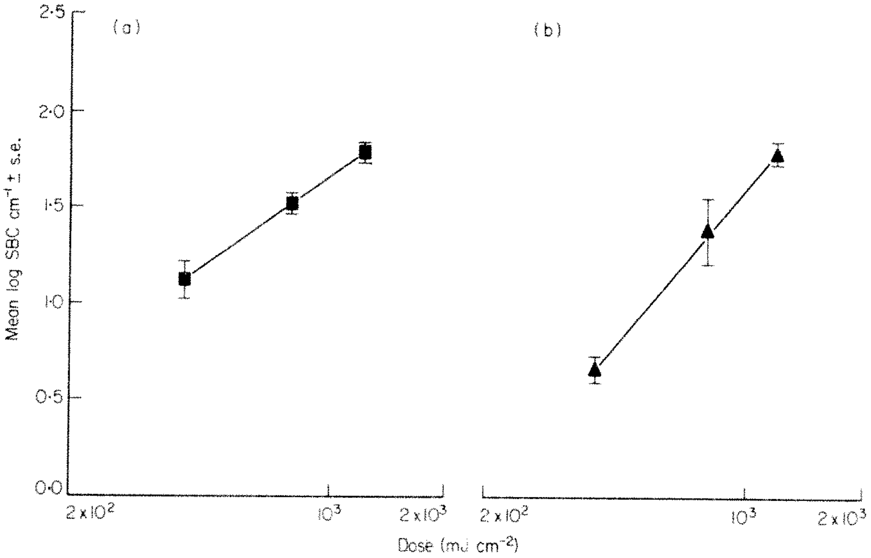

FIGURE 2. 8-MOP dose-response curve at 312·5 nm. (b) Control dose-response curve at 312·5 nm.

TABLE 1. Parameters for linear regression fits: irradiation 305–312·5 nm

λ (nm)	Treatment	Slope (α) ± s.e.	Intercept (β) ± s.e.	r
305	8-MOP	0·55 ± 0·11	0·46 ± 0·28	0·76
305	Control	0·47 ± 0·08	0·64 ± 0·21	0·81
312·5	8-MOP	1·38 ± 0·21	−2·49 ± 0·61	0·88
312·5	Control	2·36 ± 0·32	−5·48 ± 0·92	0·90

TABLE 2. Parameters for linear regression fits: irradiation 320–375 nm

λ (nm)	Number of dose points	Slope (α) ± s.e.	Intercept (β) ± s.e.	r
320	5	1·66 ± 0·20	−3·33 ± 0·59	0·87
335	4	1·02 ± 0·21	−1·40 ± 0·67	0·76
350	5	1·38 ± 0·12	−3·12 ± 0·40	0·92
365	4	1·36 ± 0·18	−3·25 ± 0·63	0·87
375	3	1·36 ± 0·31	−3·90 ± 1·24	0·81

indicating that no photosensitization has taken place (see Discussion). At 312·5 nm, although there is some evidence of 8-MOP photosensitization at the lower UVR doses, there is none in the region of the end-point. From Fig. 4, it appears that the spectral region 320–335 nm is the most effective for 8-MOP induced production of sunburn cells.

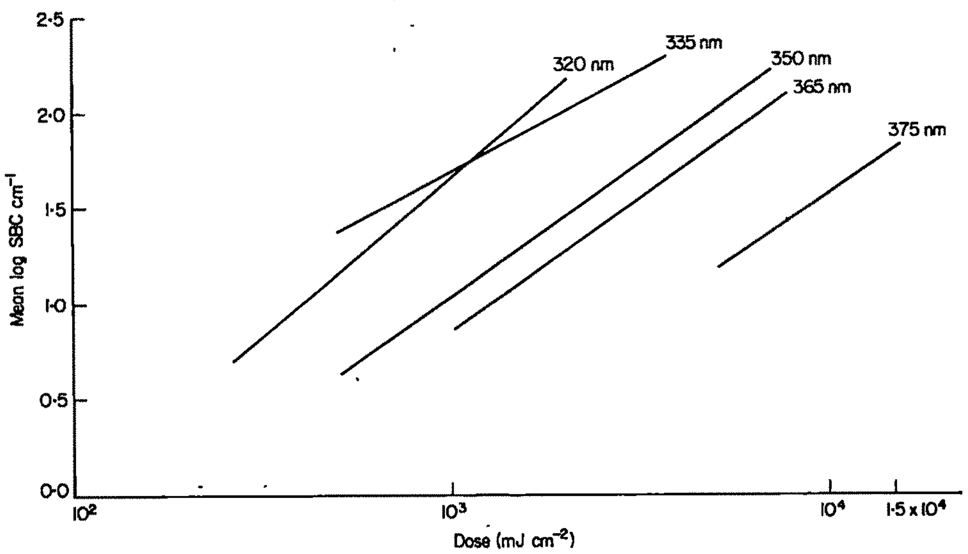

FIGURE 3. Summary of 'control corrected' UV-A dose-response curves. Pooled slope is 1·37.

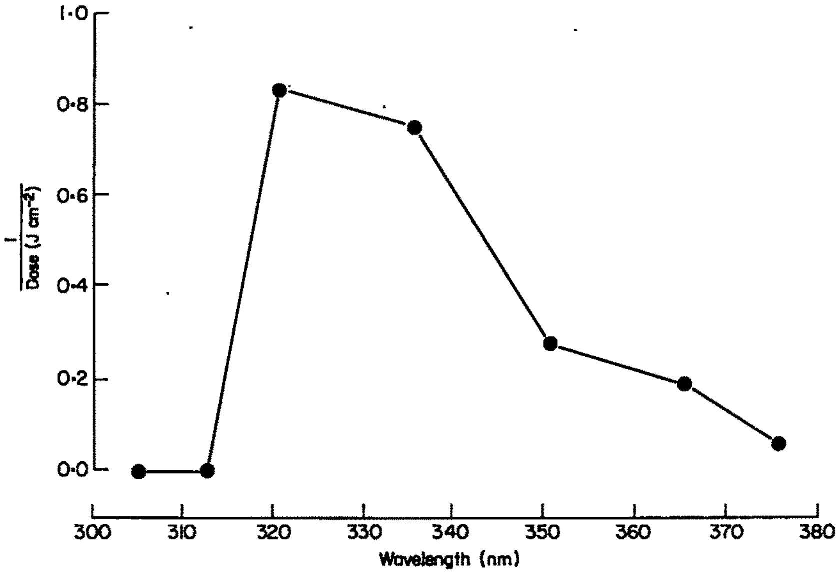

FIGURE 4. Action spectrum for 8-MOP induced sunburn cells. End-point is $\log_{10}$ 61·25 SBC cm^{-1} at 20 h post-irradiation.

DISCUSSION

The dose-response studies confirm and extend the results of Woodcock & Magnus (1976), i.e. that sunburn cell induction by UV-B and UV-A plus 8-MOP is dose related. The slopes of the dose-response curves, where 8-MOP plus UV-A were used, are not significantly different from each other; this suggests similar photochemical and/or photobiological mechanisms at these wavelengths. The dose-response curves at each wavelength have been used to compile an action spectrum, in which peak activity has been demonstrated in the region 320–335 nm.

The apparent lack of any 8-MOP induced effects with UV-B must be viewed with caution as the time courses for UVR alone and UVR plus 8-MOP may not be similar if different photobiological processes are involved. At 305 nm, the control and 8-MOP dose-response curves are virtually superimposable. This suggests similar photobiological mechanisms in both cases and that photosensitization has not taken place. However, at 312·5 nm, although no photosensitization was observed in the region of the end-point, the significantly different slopes of the control and 8-MOP curves provide evidence for different mechanisms. Exposures centred at this wavelength comprise both UV-B and shortwave UV-A, so it is interesting to note that the 8-MOP slope, i.e. 1·38, is very similar to the pooled slope of the UV-A plus 8-MOP dose-response curves, i.e. 1·37.

The sunburn cell action spectrum described here corresponds well with most other 8-MOP plus UV-A action spectra, *viz.* for growth inhibition of *C. albicans* (Owens *et al.*, 1968) and of *S. aureus* (Nakayama *et al.*, 1974); lethality in lamba and T4 bacteriophage (Suzuki *et al.*, 1977); guinea-pig eye damage (Freeman & Troll, 1969); hair growth inhibition, as a possible marker for DNA synthesis (Johnson & Bryce, 1979) and for skin erythema photosensitization (Cripps & Lowe, 1978). The disadvantage of using skin erythema as a yardstick for response, as in the earlier experiments of Buck, Magnus & Porter (1960) and Pathak (1961), who reported maximal 8-MOP sensitization near 360 nm, is that the threshold erythema dose point at each waveband has to be used for the response rather than the curve of a dose-response study. The latter method allows the crucial issue to be kept in mind, *viz.* whether the mechanism at different spectral points is similar or not.

The maximal 8-MOP sensitization values of 320–335 nm are nearer to the absorption peak of 8-MOP in ethanol, i.e. at about 300 nm, than the peaks of Buck *et al.* (1960) and Pathak (1961) at 360 nm, but they still display a definite shift to the longer wavelengths. In the case of the sunburn cell action spectrum, this could be due to a fall-off in epidermal transmittance at the shorter wavelengths, but appears not to be the only explanation as Blum (1959) and others have found that between 320 nm and 370 nm there is only a very small increase in transmission. A possible explanation for this phenomenon may be sought, assuming that the measured biological end-points may be attributed to DNA crosslinks, in the results of Dall'Acqua *et al.* (1979). These authors have suggested that crosslinking depends on two independent processes; first the formation of the 4′,5′ mono-adduct, followed by the reaction at the 3, 4 bond, but that 3, 4 mono-adducts cannot, with UV-A, further photoreact to form crosslinks. Psoralen and 8-MOP show a maximum of absorption near 300 nm; the 4′, 5′ psoralen-thymine mono-adduct has a maximum near 330 nm (Musajo & Rodighiero, 1972).

Seki, Nozu & Kando (1978) have reported that, in *E. coli*, mono-adducts were responsible for mutation and crosslinks for killing. If the hypothesis in the above paragraph were correct, 8-MOP action spectra studies in *E. coli* might prove very interesting; peak efficiency for killing might be 320–330 nm but, for provoking mutation, the action spectrum maximum might correspond with 8-MOP absorption, i.e. lie nearer 300 nm.

The primary chromophore for sunburn cell induction remains unknown. Johnson *et al.* (1972) advanced evidence for the lysosome. However, according to Hönigsmann, Wolff & Konrad (1974), epidermal lysosomes remain intact immediately after UVR injury. DNA must also be considered as a possible target. Although the sunburn cell action spectrum studies of Woodcock & Magnus (1976) did not, as might be expected from the DNA absorption spectrum, show enhanced activity in the 260 nm region but rather a plateau between 260–290 nm with a sharp fall-off to 310 nm, their results bear a close resemblance to the action spectrum obtained for thymine dimer production in mammalian skin *in vivo* (Cooke & Johnson, 1978). *In vitro* studies in mammalian cells, in which DNA may be considered a likely chromophore, have also resulted in similar action spectra curves (Rothman & Setlow, 1979; Kantor, Sutherland & Setlow, 1980). One possible explanation for the unexpectedly low activity in all these studies is that according to Deering (1962) the shorter wavelengths are more efficient at dimer monomerization. In an autoradiographic study, Brenner & Gschnait (1979) showed that, compared to histologically normal keratinocytes, relatively few (about 25%) sunburn cells showed any sign of DNA repair immediately after irradiation. However, the grain counts in the sunburn cells that did exhibit DNA repair were not significantly different from those of the normal keratinocytes. These observations are, to the best of the present authors' knowledge, the first that directly correlate DNA malfunction with the sunburn cell.

The fact that 8-MOP can promote sunburn cell induction adds support to the view that DNA may be a significant chromophore in sunburn cell induction. However, it must be borne in mind that 8-MOP also photoreacts with protein (Yoshikawa *et al.*, 1979). It is evident that much work is needed to correlate subcellular and molecular events with the pathogenesis of the sunburn cell.

The action spectrum of PUVA clearing of psoriasis has not been investigated, but the emission spectra of most of the UV-A lamps used in treatment show peaks in the region of 365 nm. If the sunburn cell is a manifestation of DNA damage it may well be that such lamps are relatively inefficient in the inhibition of DNA synthesis, the supposed rationale of PUVA. Recently, there has been concern about PUVA induced carcinogenesis (Stern *et al.*, 1979). The action spectrum of this potential hazard is unknown but should it prove to be similar to other 8-MOP plus UV-A action spectra, the speculated inefficiency of the UV-A lamps used in PUVA therapy may eventually be shown to have been fortunate. Many of the UV-A irradiance meters used in conjunction with PUVA units have peak sensitivity in the 365 nm range and little or no responsivity in the shorter UV-A. This suggests that a revision of dosimetry in this field may be logical, or at least desirable.

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Absorption of ultraviolet radiation by skin surface lipid

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SUMMARY

The UVR absorption spectrum for skin surface lipid was measured by spectrometry. Significant absorption occurred throughout the UVR range, with a maximum at 215 nm. We estimate that the lipid normally present on forehead skin is likely to reduce transmission at 300 nm by about 10%.

There is good evidence that ultra-violet radiation (UVR) plays a major role in the production of basal and squamous cell cancers of the skin (Emmett, 1973) and UVR is also necessary for the cutaneous production of vitamin D_3 (Holick *et al.*, 1977). The dose-response curves for carcinogenesis and D_3 production by UVR in human skin are unknown, though several workers have attempted to correlate the observed or estimated environmental UVR doses with the observed skin cancer rates (Scott, 1975; Fears, 1976; Beadle, Pingstone & Leach, 1978) or plasma vitamin D_3 levels (Beadle, 1977; Beadle, Burton & Leach, 1980). It is generally assumed in such calculations that the non-reflected incident radiation that reaches the skin is absorbed by the epidermis, but it occurred to us that the skin surface lipid might absorb a significant proportion of the incident irradiation. We here report a study which shows that skin surface lipid does indeed absorb UVR, and it may therefore protect against skin cancer and diminish the cutaneous production of vitamin D_3.

METHODS

Skin surface lipid samples were obtained from each of seven normal volunteers (aged 13–32 years) by immersing the scalp and hair in a bowl of Analar ether for a few minutes and then filtering the ether to remove hair and squames (Burton, 1970). The ethereal solution was then distilled at 37 °C, and when sufficiently concentrated it was transferred to a stoppered glass vial. The distillation flask was rinsed with ether and the washings were added to the vial. Ether was finally removed from the vials by evaporation under nitrogen at 37 °C, and the samples were stored at −20 °C until needed.

To measure the absorption spectrum, we accurately weighed about 0·0075 g from each lipid sample and dissolved this in 4 ml hexane. The solution concentration was chosen to give a suitable peak absorption under the experimental conditions. The absorption spectrum between 200 and 400 nm was recorded for each sample with a Perkin-Elmer 24 double-beam spectrophotometer, using UV-matched cells of 10 mm path length. Pure hexane was placed in the reference cell.

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RESULTS

A typical example of the UV absorption spectrum by the skin surface lipid is shown in Fig. 1. In every subject, the maximum absorption occurred at a wavelength of about 215 nm with transmission being well below 1% at the concentrations we used. Even in the normal 'sunburn range' of 290–310 nm the skin lipid absorbs about half of the incident radiation, and significant absorption occurs throughout the UV range.

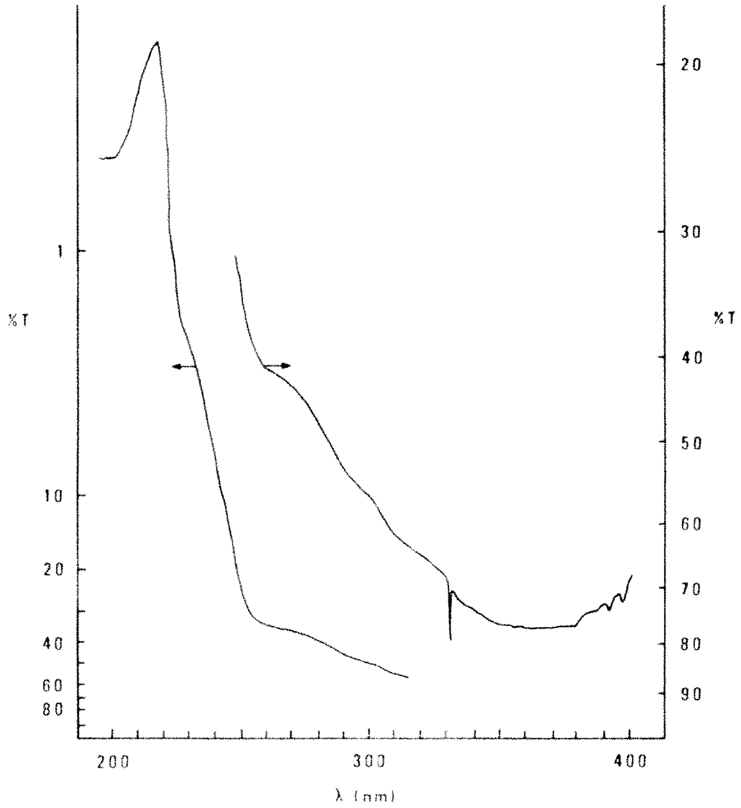

FIGURE 1. Percentage transmission (%T) of UVR of different wavelengths (λ) by skin surface lipid derived from the scalp of a male subject, aged 28 years. The right hand curve is part of the left hand curve run at a higher sensitivity. Lipid concentration 1·73 mg/ml.

Transmission at 300 nm was 47±12% at a mean concentration of 1·835±0·144 mg/cm^3. Since the path used was 1 cm this would correspond to a mean absorbing column of 1·835 mg lipid/cm^2 of skin.

DISCUSSION

Skin surface lipid is derived mainly from sebaceous glands with a small contribution from the keratinizing epidermis (Greene *et al.*, 1970). There may be large differences in skin surface lipid composition between individuals, but in each subject the sebum excretion rate and the lipid composition remain fairly constant from day to day (Downing, Strauss & Pochi, 1969).

The normal rate of sebum excretion on the adult forehead varies from a minimum value of approximately 0·2 $\mu g/cm^2/min$ in elderly females to a maximum of 1·3 $\mu g/cm^2/min$ in young men (Burton *et al.*, 1975). Most people wash on arising each morning and we may assume that by midday they will have accumulated lipid on their skin surface for about 3–6 h. The total quantity of lipid on the forehead by midday, when natural ultra-violet irradiation is at its maximum, is likely therefore to be somewhere between 36 $\mu g/cm^2$ (for an elderly woman who washed at 9 am) and 468 $\mu g/cm^2$ (for a young man who washed at 6 am) since the contribution from epidermal lipid synthesis is relatively trivial.

The present results indicate that transmission at 300 nm at the lower level of surface lipid would be about 98·5 ± 0·7%, while at the higher value the transmission would decrease to 82·0 ± 8%. Thus even at the levels likely to be found on normal skin, significant absorption of UVR by the skin surface lipids will occur. Seborrhoea may thus tend to protect against skin cancer and it may also decrease vitamin D production by the skin.

Several workers have attempted to relate the environmental UVR dosage to the reported incidence rates of skin cancer, and mathematical models have been used to predict how a change in UVR might affect this incidence rate. Our results have shown that skin surface lipid adds yet another variable which will need to be taken into account in such calculations.

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Herpes gestationis and anti-HLA immunization

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SUMMARY

The lymphocytotoxicity technique at +15°C and +37°C was used to identify anti-HLA and cold lymphocytotoxic antibodies in sera from two women with herpes gestationis. The degree of severity of the illness was different in the two cases. The more serious case (widespread cutaneous involvement, repeated fetal deaths) had a very powerful anti-HLA-A9+anti-HLA-B5 antibody (titre 1/1000); the other had less severe skin lesions; she had an anti-HLA-B13 antibody (titre was 1/256). Both cases had cold lymphocytotoxic auto-antibodies.

Herpes gestationis (HG) is a vesiculo-bullous rash of pregnancy associated with severe itching and tends to recur at each pregnancy. It is a rare disease, occurring in 1 in 3000 to 1 in 4000 pregnancies (Russell & Thorne, 1957). Both clinically and histologically herpes gestationis resembles bullous pemphigoid, dermatitis herpetiformis and erythema multiforme. Recent work seems to show that herpes gestationis is more closely related to bullous pemphigoid, particularly with regard to the deposition of complement and immunoglobulins on the basement membrane (Bushkell, Jordon & Goltz, 1974; Hönigsmann *et al.*, 1976; Yaoita, Gullino & Katz, 1976; Carruthers, Black & Ramnarain, 1977; Harrington & Bleehen, 1979).

CASE HISTORIES

Case 1

Mrs M. Fatima, aged 40, gravida IX, para IV. Two reports of her case have already been published (Bermont *et al.*, 1968; Heid *et al.*, 1973). Her case history was complex:

1959: normal delivery, a girl now aged 19 (in Algeria).
1960: premature delivery, at the 32nd week, of a girl who died on the 5th day (in Algeria).
1962: premature delivery, at the 36th week of a girl who died on the 10th day (in Algeria).
1963: intra-uterine death of a boy at the 32nd week (in Algeria).
1967: normal delivery of a boy, now aged 12 (in France).
1968: intra-uterine death of a boy at the 32nd week.

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1969: normal delivery of a girl, now aged 10.
1970: intra-uterine death of a boy at the 28th week.

The earliest lesions, typical of herpes gestationis, appeared in the 32nd week of the second pregnancy in 1960 and their overall duration was 20 days. In 1962, during the 3rd pregnancy, the lesions were not confined to the skin but also spread to the mucous membranes.

During subsequent pregnancies cutaneous and mucosal signs of the disease appeared at increasingly earlier dates and were progressively more severe. The lesions disappeared after the puerperium but later became persistent; this persistence, from January 1970 to March 1972, seemed to be related to the taking of an oral contraceptive and it ceased when oral contraception was replaced by insertion of an intra-uterine device. The skin condition was satisfactory from 1972 to March 1978. The patient was admitted to hospital in 1979 during her 9th pregnancy; in the 12th week an urticaria-type rash was observed with circinate erythematous plaques on which vesicles and bullae soon formed (Fig. 1). The rash started in the peri-umbilical region and on the inner surface of the thighs and spread rapidly. Apart from obesity and oedema of the lower limbs, her general condition was otherwise satisfactory.

Routine blood tests were satisfactory except for an eosinophilia (1507 to 2050/mm^3). There was a fall in the total serum protein level to 53 g/l ($n = 72 \pm 10$ g/l); there was a decrease in albumin alpha-2 macroglobulin and IgG. The level for IgG was 8·3 g/l ($n = 9 - 14$ g/l). Haptoglobin was increased. The other fractions were normal.

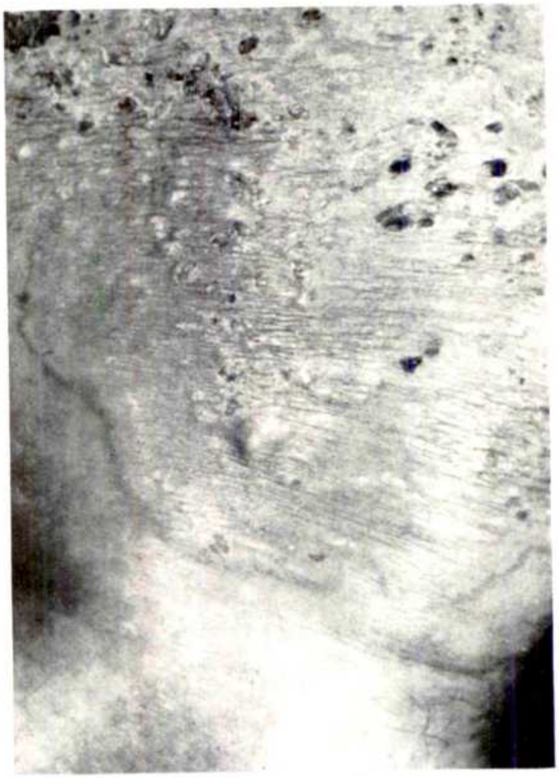

FIGURE 1. Urticarial circinate erythematous plaque with vesicles and bullae on the inner surface of the right knee.

Direct IF findings in a biopsy of skin from a lesion showed linear and continuous deposition at the B.M. zone with an anti-complement serum (+ + +); no deposits of IgA, IgG and IgM. Healthy skin showed linear and continuous deposition at the B.M. zone with an anti-complement serum (+ +): no deposits of IgA, IgG and IgM.

Findings for total complement and for C3 were normal. No anticomplementary effect was found in the serum. No circulating immune complexes were detected (PEG-C4 test). No circulating anti-B.M. antibody was found. Blood group: O, Rh +.

Oral (Cortancyl, 60 mg daily) and topical corticosteroid treatment was started to relieve the widespread very itchy vesiculo-bullous rash. This gave rapid but transient relief, and, in spite of treatment, a more severe outbreak seems to have preceded intra-uterine death by several days. Pregnancy was terminated in the 18th week; a Syntocinon drip was started on 5 June 1978 and the uterus evacuated. The male fetus weighed 230 g, length 21 cm. The ears were situated at an abnormally low level. Identification of other abnormalities was impossible due to maceration.

Following this, widespread skin involvement of the lower limbs made it necessary to increase the dose of steroid (Cortancyl 90 mg).

Case 2

Mrs A. Aicha, 32 years, gravida X, para IV. Previous history:

1968: normal delivery of a girl in Morocco.
1970: normal delivery of a girl in Morocco.
1972–73: 4 spontaneous abortions.
1976: normal delivery of a girl in Morocco.
1977: spontaneous abortion.
1978: extra-uterine pregnancy—left salpingectomy.

The patient was first seen at the Dermatology Clinic in March 1979. At that time she was in the 32nd week of her 10th pregnancy. Two weeks prior to admission, in the 30th week of the current pregnancy, she first complained of itching and the typical rash appeared (Fig. 2). There was no history of itchiness or rash in previous pregnancies.

Histological examination was typical for herpes gestationis.

Direct IF findings in a biopsy of affected skin (from the anterior surface of the left arm): the basement membrane was very lightly outlined with an anti-complement serum (C3 positive at 1/8). Absence of IgA and IgG deposits. Healthy skin showed the basement membrane lightly outlined with an anti-complement serum (C3 positive at 1/10); an absence of IgA and IgG deposits.

Electrophoresis and immunoelectrophoresis showed a moderate hypoalbuminaemia, with hyper alpha 1, hyper alpha 2 and an increase in beta-I-globulins at the expense of the transferrin arc; the immunoglobulins were normal. Blood group: A2, Rhesus +.

MATERIALS AND METHODS

HLA typing of the two women, their husbands and children was carried out using the microlymphocytotoxic technique (Terasaki & MacClelland, 1964). Sera collected from both patients on different occasions were studied at +15°C and +37°C on their own lymphocytes, on those of their husband and children and on lymphocytes of 70 HLA typed panel lymphocytes. Antibody detection at +37°C was aimed at demonstrating anti-HLA antibodies; at +15°C at identifying cold lymphocytotoxic antibodies. The methods used to demonstrate these antibodies have been described earlier (Mayer, Falkenrodt & Tongio, 1974).

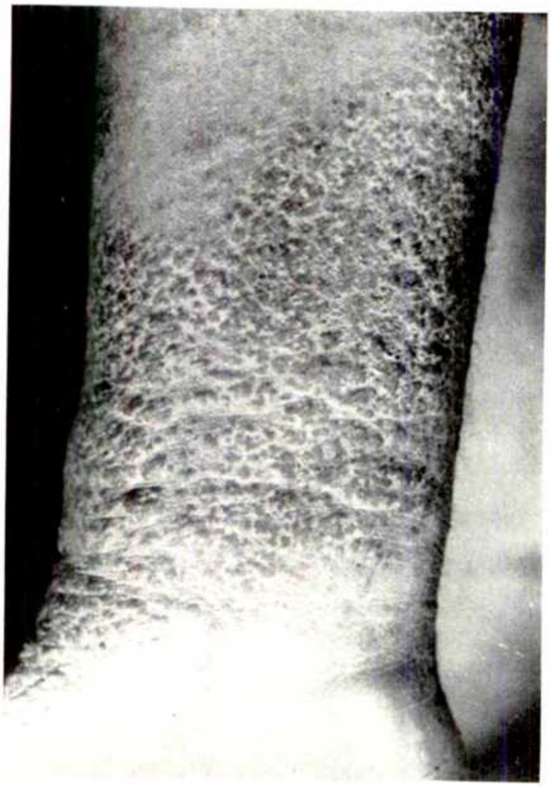

FIGURE 2. Case 2. Vesicular rash on the anterior surface of the lower third of the left forearm.

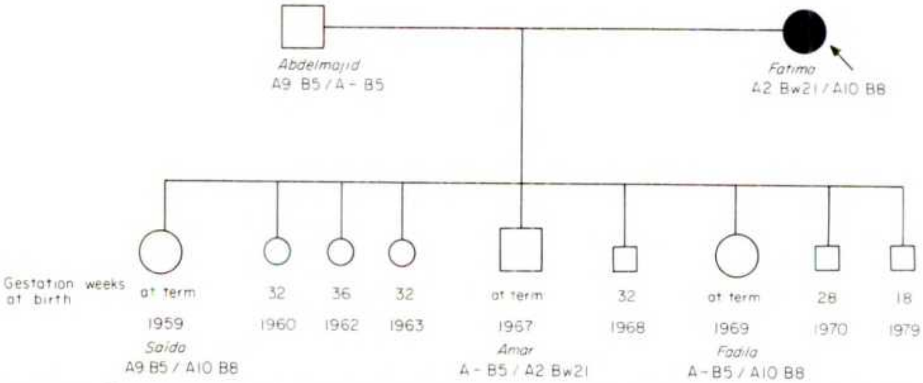

FIGURE 3. Genotype of the Maz's family. ●, Proposita; □, healthy man; ○, healthy woman; ○, neonatal death; □, intrauterine death.

RESULTS

HLA genotypes for both families are given in Figs 3 and 4.

Antibody titres in serum from these patients tested on autolymphocytes and on lymphocytes from their husbands and children are shown in Tables 1 and 2. In both cases the patient's serum was found to contain anti-HLA antibodies. Tested on the husband's lymphocytes the anti-HLA antibody

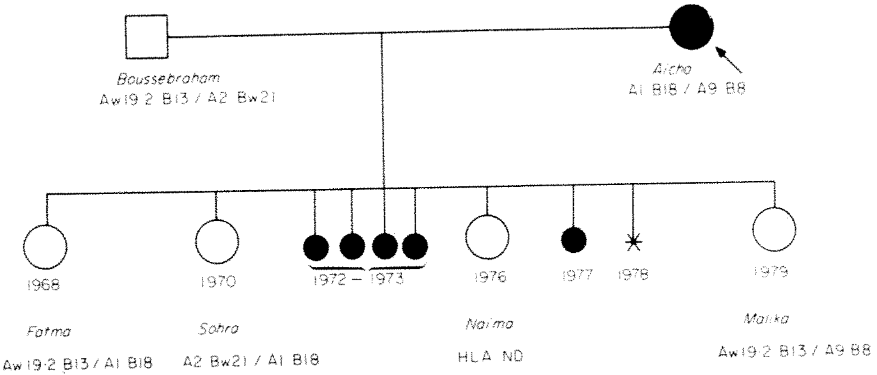

FIGURE 4. HLA genotype of the AHR's family. *, Total pregnancy; ND, not done; ●, proposita; ○, healthy woman; ☐, healthy man; •, spontaneous abortion.

TABLE 1. Titre of HLA and cold auto-lymphocytotoxic antibodies in Mrs MAZ's serum

Tested on the following lymphocytes	Date of serum	Tested at +15°C	Tested on +37°C
MAZ Fatima	23.10.69	++ at 1/1	—
	25.06.71	+++ at 1/1	—
	13.04.79	++ at 1/1	—
	23.04.79	++ at 1/1	—
	25.04.79	++ at 1/1	—
MAZ Abdelmajid 'husband'	23·10·69	++++ at 1/1000	++++ at 1/1000
	25.06.71	+++ at 1/1000	++++ at 1/1000
	13.04.79	++++ at 1/1000	++++ at 1/500
	23.04.79	? ?	++++ at 1/64
	25.04.79	++++ at 1/128	+++ at 1/128
MAZ Saida 'child'	23.10.69	++++ at 1/2000	+++ at 1/500
	25.06.71	++++ at 1/2000	++++ at 1/500
	13.04.79	++++ at 1/1000	++++ at 1/500
	23.04.79	++++ at 1/256	+++ at 1/128
	25.04.79	+++ at 1/256	++++ at 1/256
MAZ Amar 'child'	23.10.69	++++ at 1/1000	++++ at 1/1000
	23.01.71	++++ at 1/1000	++++ at 1/1000
	13.04.79	++++ at 1/1000	++++ at 1/1000
	23.04.79	++++ at 1/1000	++++ at 1/32
	25.04.79	+++ at 1/128	++++ at 1/128
MAZ Fadila 'child'	23.10.69	++++ at 1/1000	+++ at 1/500
	25.01.71	++++ at 1/1000	++++ at 1/1000
	13.04.79	++++ at 1/1000	+++ at 1/500
	23.04.79	++++ at 1/128	++++ at 1/128
	25.04.79	++++ at 1/128	++++ at 1/128

titre was 1/1000 for Mrs M and 1/8 for Mrs A. Serum from Mrs M studied at 1/500 on the panel cells contained an anti-A9+B5 antibody. Undiluted, this serum was found to be polyspecific. Serum from Mrs A contained anti-B13, anti-Aw19·2 and anti-Bw21 antibodies as well as anti-B12+B40+B17+B15 antibodies developed by cross reactions. When tested at +15°C on lymphocytes from their husbands and children serum from both patients gave a positive reaction with titres close to

TABLE 2. Titre of HLA and cold auto-lymphocytotoxic antibodies in Mrs AHR's serum

Tested on the following lymphocytes	Date of serum	Tested at +15°C	Tested at +37°C
AHR Aicha	17.04.79	+++ at 1/2	—
	15.05.79	+ at 1/2	—
	11.09.79	++ at 1/2	—
	19.10.79	+ at 1/2	—
AHR Boussebraham 'husband'	27.04.79	++++ at 1/2	++++ at 1/8
AHR Fatma 'child'	27.04.79	++++ at 1/2	++++ at 1/2
AHR Sohra 'child'	27.04.79	++++ at 1/2	++++ at 1/2

those observed at +37°C. This was very likely due to anti-HLA antibodies as we have demonstated earlier (Mayer *et al.*, 1974) that anti-HLA antibodies were clearly visible at +15°C. Tested at +15°C on autolymphocytes from each of the two patients, in both cases their cells reacted positively with undiluted serum or serum diluted to 1/2. Thus both anti-HLA antibodies and cold lymphocytotoxic antibodies were present in the serum of these two patients.

DISCUSSION

Reunala *et al.* (1977) reported a case of herpes gestationis in a primiparous patient who developed an anti-HLA antibody with a titre of 1/100. In the case of Mrs M, we observed an exceptionally high titre of anti-HLA antibody for a multigravida (1/1000).

In the case of Mrs AHR, tested on the lymphocytes of the panel, the anti-B13 antibody was 1/256 when tested on 3 B13 panel lymphocytes whereas it was only 1/8 when tested on the husband's lymphocytes. This raised the question of the presence in the serum of an additional blocking antibody specifically directed against the husband's and children's antigens.

Clinical aspects of the disease varied in these two patients: Mrs M, who had a higher anti-HLA antibody titre than Mrs A, was much more seriously affected.

Reunala *et al.* (1977) observed that in their patients 10% of primipara developed anti-HLA antibodies with titres varying usually between 1/1 and 1/8. These authors suggested that the high titre of anti-HLA antibodies developed in the HG patients could have a possible pathogenic role especially in the formation of immune complexes which could interfere with placental function and explain the high rate of abortion and still-births observed in these patients.

In our cases a high titre of anti-HLA antibodies was also observed. We could not rule out the titre of these antibodies being the result of multiple pregnancies. However, in studying cases with from one to greater than five confinements, only 1% of sera had a titre of 1/128. Unfortunately it was impossible to find in our case records non-HG sufferers with such a history of abortions and repeated fetal deaths in order to study their anti-HLA immunization and compare them with the two patients. Nevertheless, the fact that Reunala *et al.* reported a high antibody titre in a primipara could suggest that this high titre was part of the disease. A theory worth checking would be that these high titres resulted from a dysfunction or deficit of T suppressor cells; this would cause disruption of the humoral response. It would also be of interest to find out if these patients had abnormally high titres of antibodies against erythrocytic antigens, if there is an incompatibility for these antigens between the mother and the child.

However, no abnormality was found in cold autoantibody titres. Indeed, these antibodies were detected with comparable titres in anti-HLA sera (Mayer, Hauptmann & Tongio, 1976) from patients with previously normal pregnancies.

Both women in our study had HLA-B8. In the case described by Reunala *et al.*, the patient did not have this antigen. It would be of interest if other centres with similar cases carried out HLA typing. This would then enable these results to be compared with those in dermatitis herpetiformis where the B8 antigen is significantly increased (Reunala *et al.*, 1976) and those in bullous pemphigoid were no association with the HLA system has been observed (Ahmed *et al.*, 1977) although too few cases of the latter disease have been studied.

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The association between lichen sclerosus et atrophicus and HLA-B40

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SUMMARY

Fifty Caucasian females with lichen sclerosus et atrophicus were investigated to determine their HLA types. HLA-B40 was the only antigen found to be significantly more common in these patients when compared with a control population.

Recent work (Harrington & Dunsmore, 1981) has shown that patients with lichen sclerosus et atrophicus have an increased incidence of other auto-immune disorders when compared with a control population. This finding prompted an investigation to determine whether HLA antigens, such as B8, which are more common in patients with auto-immune diseases (Bodmer, 1978), are also more common in LSA.

METHODS

Fifty Caucasian females with histologically proven LSA were tissue typed for 27 HLA, A and B antigens. The incidence of the HLA antigens was compared with the distribution of these antigens in 3000 local blood donors.

HLA typing was performed using a microlymphocytotoxic test (Gelsthorpe & Doughty, 1971), each specificity being defined by at least two antisera.

RESULTS

Using the relative incidence analysis of Woolf (1955), as discussed by Svejgaard *et al.* (1974), B40 was the only antigen found to be significantly more common in patients with LSA ($P<0.003$), being present in fourteen of the fifty patients (28%) and 404 of the 3000 controls (13.4%). However, after correcting for the number of antigens tested, the significance level drops to $P<0.081$ which is not statistically significant, but strongly suggestive for other independent studies.

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DISCUSSION

The increased incidence of HLA-B40 in LSA is a new finding. The only other diseases known to be definitely associated with B40 are alcoholic liver disease with cirrhosis (Bell & Nordhagen, 1978) and oesophageal carcinoma in Turkish mongols (Simons & Amiel, 1978). One study has found B40 to be a risk factor for young insulin dependent diabetics (Ludvigsson, Safwenberg & Heding, 1977). The increased incidence of B40 in patients with LSA suggests that this antigen may be in linkage disequilibrium with immune response genes controlling the susceptibility to both LSA and other auto-immune diseases.

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An investigation into the incidence of auto-immune disorders in patients with lichen sclerosus and atrophicus

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SUMMARY

Patients with lichen sclerosus and atrophicus have an increased incidence of organ specific antibodies, and these patients and their relatives have a significantly higher incidence of associated autoimmune diseases than a control population. The incidence of other auto-immune diseases in patients with lichen sclerosus is highest when the onset is between the ages of 41 and 60 years, but is not related to the duration or site of the lesions.

These findings suggest an immunological basis for the disease and indicate that the patients should be investigated and followed up to detect the existence of other auto-immune disorders.

INTRODUCTION

Lichen sclerosus and atrophicus (LSA) is an uncommon disease, of unknown aetiology, in which small white areas on the skin may be associated with an atrophic condition of the vulva and perianal skin. In 1971 Wallace reported that ten of his 380 patients with LSA also had vitiligo and suggested that this association was probably significant. In 1974, Goolamali *et al.* reported that 40% of their patients had antibodies to thyroid cytoplasm and 44% had gastric parietal cell antibodies.

This investigation was carried out to determine whether there is a definite association between LSA and other auto-immune disorders.

PATIENTS AND METHODS

Over a period of 4 years, fifty Caucasian females with histologically proven LSA have been investigated. They were compared with fifty age-matched controls who were attending the skin clinic for minor complaints and were investigated concurrently. The age of presentation of patients ranged from 8 to 85 years, mean 54·86 (s.d. 17·62, s.e. 2·49). The age at onset of symptoms was from 6 to

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80 years, mean 49·44 (s.d. 18·97, s.e. 2·68). The duration of symptoms varied enormously from 2 months to 30 years, mean 5·12 years (s.d. 6·3, s.e. 0·89). Thirty-two of the patients had ano-genital LSA: sixteen just of the vulva and sixteen of the vulva and peri-anal skin. Ten had ano-genital and extra-genital lesions and eight had just extra-genital LSA.

All the patients and controls were interrogated, examined and investigated for pernicious anaemia, hypothyroidism, thyrotoxicosis, alopecia areata, diabetes mellitus and vitiligo. An enquiry was made into whether any first, second or third degree relative suffered from any of these conditions. If necessary hospital records were obtained to verify diagnoses. Other investigations performed were: anti-nuclear factor, smooth muscle antibody, anti-mitochondrial antibody, thyroid antibodies, gastric parietal cell and intrinsic factor antibodies.

RESULTS

The first striking finding of an exploratory analysis of the data was that seventeen of the patients (34%) with LSA were suffering or had suffered from at least one other auto-immune disease compared with only two in the control group (Table 1). A χ^2 goodness of fit test showed that this difference is highly significant ($P<0{\cdot}001$). In fact four of the seventeen patients had two other diseases—two had pernicious anaemia and hypothyroidism, one had pernicious anaemia and diabetes and one had thyrotoxicosis and alopecia areata. This gives a total of twenty-one auto-immune diseases in the patient group, and this survey led to the diagnosis of one new case each of hypothyroidism and diabetes. However, as can be seen in Table 1, in this small group, when the conditions are analysed individually by use of exact Fisher-Irwin tests, pernicious anaemia is the only disorder which is significantly more common in the patients than in the controls ($P<0{\cdot}03$).

TABLE 1. Presence of other disorders

	Patients (50)	Controls (50)	*P* value
With at least one disorder	17	2	$P<0{\cdot}001$
Individual disorders			
Pernicious anaemia	5	0	$P<0{\cdot}03$
Hypothyroidism	2	0	NS
Thyrotoxicosis	4	1	NS
Alopecia areata	5	1	NS
Diabetes	3	0	NS
Vitiligo	2	0	NS

NS = not significant.

Statistical analysis using the χ^2 test was performed to determine whether the age of onset, duration of LSA or the site of the lesions were related to the incidence of other auto-immune diseases. The age of onset was divided into three groups—under 40 years (12 patients), 41–60 years (20 patients) and 61–80 years (18 patients). There was a significantly greater incidence ($P<0{\cdot}05$) of other conditions in the twenty patients whose onset of LSA was between the ages of 41 and 60 years. Whether the patients had their LSA for under or above 5 years made no significant difference to the presence of other diseases, and the site and extent of the lesions bore no significant relationship to the presence or absence of other auto-immune disorders. There were no other conditions which appeared to be more common in LSA patients, but two patients in this group had squamous cell carcinomata of the peri-anal skin.

Enquiry into the family histories revealed that eighteen patients had relatives suffering from a total of thirty auto-immune disorders (Table 2). Nineteen of these were in first degree relatives, nine in second degree and two in third degree relatives. Only seven controls had relatives with a total of eight other conditions and this overall difference is significant ($P<0{\cdot}025$). When the conditions are analysed individually again using the Fisher–Irwin test pernicious anaemia and diabetes are significantly more common in relatives of patients with LSA than in relatives of controls. The age of onset, duration of symptoms and site of lesions bore no significant relationship to the incidence of auto-immune diseases in the families of patients with LSA; one patient had a sister who also had LSA.

The results of investigations for tissue antibodies (Table 3) show that thirty-seven patients had at least one tissue antibody compared with ten controls, a highly significant difference ($P\leqslant 0{\cdot}001$).

Taken individually, thyroid microsomes ($P<0{\cdot}005$), thyroglobulin antibody ($P<0{\cdot}005$), gastric parietal cell antibody ($P<0{\cdot}001$) and intrinsic factor antibody ($P<0{\cdot}003$) were significantly more common in the patient group. Sixteen patients had more than one organ specific antibody. Twenty-

TABLE 2. Family History

	Patients (50)	Controls (50)	*P* value
With at least one relative suffering from an auto-immune disease	18	7	$P<0{\cdot}025$
Individual disorders			
Pernicious anaemia	8	0	$P<0{\cdot}003$
Hypothyroidism	3	2	NS
Thyrotoxicosis	2	1	NS
Alopecia areata	2	1	NS
Diabetes	13	4	$P<0{\cdot}02$
Vitiligo	2	0	NS

NS = not significant.

TABLE 3. Tissue antibodies

	Patients (50)	Controls (50)	*P* value
With at least one tissue antibody	37	10	$P\leqslant 0{\cdot}001$
Individual antibodies			
Antinuclear factor	15	7	NS
Smooth muscle antibody	2	1	NS
Thyroid microsomes	12	1	$P<0{\cdot}005$
Thyroglobulin	12	1	$P<0{\cdot}005$
Tanned red cell titre	2	0	NS
Gastric parietal cell antibody	17	2	$P<0.001$
Intrinsic factor antibody	5	0	$P<0{\cdot}03$

NS = not significant.

three patients had two positive thyroid antibodies, but only four of these had thyroid disease—two hypothyroidism and two thyrotoxicosis. However according to laboratory standards, the titres of thyroid antibodies were higher than those found in normal population samples. The five positive intrinsic factor antibodies were in the five patients with pernicious anaemia and twelve others had positive gastric parietal cell antibodies. The positive antibodies were evenly distributed throughout the age groups and were not, as might have been expected, more common in the older patients. The presence of tissue antibodies was not related to the duration or site of LSA.

DISCUSSION

This survey has shown that patients with LSA have a significantly higher incidence of associated auto-immune diseases than a control population. Thus these patients should be screened and followed up to detect such disorders. The aetiology of LSA remains unknown, but this proven association with other auto-immune diseases does suggest that the condition may have an immunologically mediated pathogenesis.

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Case Reports

Idiopathic pyoderma gangrenosum and impaired lymphocyte function: failure of azathioprine and corticosteroid therapy

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SUMMARY

A 58-year-old man with chronic 'idiopathic' pyoderma gangrenosum, recurrent secondary staphylococcal infection, nail candidiasis, markedly impaired lymphocyte function and a serum blocking factor is described. Despite an initially favourable response to immunosuppressive therapy, the patient later relapsed whilst on high dose systemic corticosteroids and azathioprine. Immune function remains abnormal 3 years after cessation of azathioprine. Attempted immunostimulation with *Corynebacterium parvum* proved unsuccessful. A striking but transient increase in lymphocyte function followed parenteral iron therapy for an unexplained iron-deficiency anaemia. Gradual reduction in prednisone dosage to 5 mg daily has coincided with a period of clinical resolution and modest improvement in lymphocyte reactivity. Prolonged immunosuppressive therapy may be inadvisable in chronic idiopathic pyoderma gangrenosum associated with defective cellular immunity.

Holt *et al.* (1980) described two types of pyoderma gangrenosum: an acute progressive type in which ulcers rapidly enlarged until arrested by treatment, and a more chronic form in which lesions extended slowly and then gradually healed spontaneously. The poorly defined natural history of the untreated disease and the absence of controlled clinical trials make the assessment of therapeutic benefit difficult. We report the results of a 7 year follow-up of a patient with chronic pyoderma gangrenosum without any demonstrable underlying disease, previously documented as having responded to a combination of high dose corticosteroid and azathioprine therapy (August & Wells, 1974). This treatment subsequently failed to control the pyoderma gangrenosum, and azathioprine was discontinued in 1977, when the patient's immune function was markedly impaired. The present report indicates that claims of therapeutic benefit in pyoderma gangrenosum which are not based on prolonged follow-up should be interpreted with caution. The literature on pyoderma gangrenosum in relation to immunological studies is discussed.

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CASE REPORT

The patient, a 58-year-old male, developed pyoderma gangrenosum in 1972 following a suprapubic prostatectomy for benign prostatic hyperplasia. Extensive investigation in 1973 failed to reveal an underlying cause, and oral iron therapy, transfusion, intralesional steroid injections and salazopyrine were all ineffective. From August 1973 to March 1977 the patient received a combination of azathioprine 150 mg and prednisone 30–40 mg daily. During this period disease activity lessened considerably and individual lesions could be controlled with local treatment including radiotherapy. Azathioprine was, however, discontinued in April 1977 as a result of failure to control a severe exacerbation of his disease and because *in vitro* lymphocyte function was shown to be severely impaired. An 8-week trial of clofazimine, 300 mg daily (Michäelsson *et al.*, 1976), in May and June 1977 produced no benefit. Abnormal fibrinolysis was noted in July 1977, the dilute blood clot lysis time being 1020 minutes (normal 240–280) and the clot weight fibrinogen 566 mg% (normal 250–350). Stanozolol 10 mg daily corrected this abnormality; there was slow clinical improvement, prednisone therapy being maintained at 40 mg daily. In May 1978 the patient sustained steroid-induced collapse of several thoracic and lumbar vertebrae. Subsequent extensive and refractory cutaneous ulceration with recurrent staphylococcal superinfection necessitated his admission in August 1978.

EXAMINATION (AUGUST 1978)

Numerous areas of 'cigarette paper' atrophy were scattered over the trunk and lower legs at the site of healed lesions. Radiation changes were present on the left scapular and right pectoral areas. Active pyodermatous lesions were present on the left buttock, right thigh, upper outer arms, left upper back (Fig. 1) and right pectoral regions, showing necrotic ulceration, bluish undermined margins and an erythematous halo. There was a symmetrical nail dystrophy and kyphoscoliosis.

INVESTIGATIONS

The following were normal. Polymorphonuclear leukocyte and platelet count, serum B12 and folate, direct Coombs' test, cryoglobulins, sucrose lysis test, serum and urine immuno-electrophoresis, marrow morphology, serum calcium, liver function tests, fasting glucose, TPHA and VDRL, rheumatoid factor, ANF, smooth muscle antibody, MSU, intravenous pyelography, mediastinal CAT scan, tests for faecal blood loss, jejunal biopsy, rectal biopsy, barium meal and follow through, barium enema and colonoscopy. Culture of biopsy material from early unbroken pustules was negative for bacteria, fungi and viruses.

The following were abnormal. Haemoglobin 11·6 g/dl, ESR 93 mm/hr (Westergren), serum iron 12·6 μmol/l (normal 13–32), serum UIBC 74·1 μmol/l (normal 45–70), absent stainable bone marrow iron stores, urea 9·9 μmol/l (normal <7·0) and creatinine clearance 44 ml/min. Skin swabs taken from ulcerated areas grew *Staphylococcus aureus* resistant to penicillin on repeated occasions, though primary lesions (unbroken pustules) were sterile. Skin biopsies showed non-specific ulceration bordered by superficial abscess formation and areas of deeper, more chronic inflammation; no organisms were seen. Nail clippings grew *Candida parapsilosis*.

Immunological function. There was cutaneous anergy to Candida, PPD and Trichophytin. Serum IgM was normal but IgG was slightly reduced at 5·8 g/l (normal 6–16), and IgA was low at 0·75

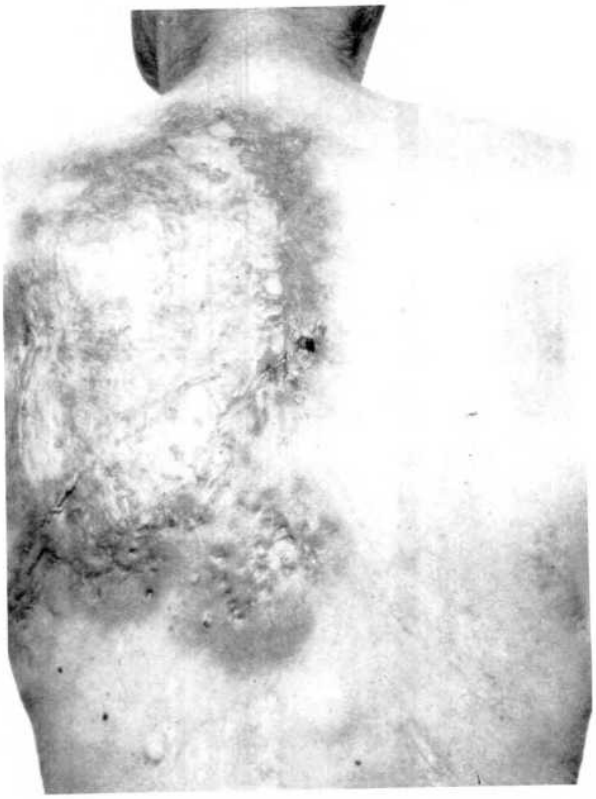

FIGURE 1. Active pyoderma gangrenosum on the left upper back. Note radiation changes in the left scapular region.

g/l (Normal 1·2–4·0). Serum complement levels (CH_{50}, C4 and C3) were normal and circulating immune complexes (Harkiss & Brown, 1979) were not detected.

Polymorphonuclear and monocyte migration, assessed by a raft technique (Addison & Babbage, 1976) was found to be normal. The neutrophil phagocytosis index (Bridges, Dasilva & Valdimarsson, 1980) was reduced at 2·1 (normal 3·1–4·9), but intrinsic ability of neutrophils to kill *Candida albicans* was normal. Complement dependent opsonization (Yamamura & Valdimarsson, 1978) was also normal. There was a lymphopenia of 714/μl of blood (normal 1500–3500) with a normal T : B cell ratio. The patient's lymphocytes failed to respond to phytohaemagglutinin (PHA), conconavalin (Con A), poke-weed mitogen, and PPD in a thymidine incorporation assay (Valdimarsson & Boler, 1981) (Fig. 2). The patient's plasma, present throughout the culture period, significantly inhibited the PHA reactivity of normal control lymphocytes, with reduction in the stimulation index from eighty for control cells in 10% of autologous plasma to forty-eight for control cells in 10% of patient plasma. However, the patient's cells remained hyporesponsive when cultured in control plasma after extensive washing. Studies on lymphokine production were not performed.

MANAGEMENT AND PROGRESS

The cutaneous lesions failed to respond to simple local wound toilet, intermittent courses of flucloxacillin, prednisone 30 mg daily and stanozolol 10 mg daily. In an attempt to improve the patient's

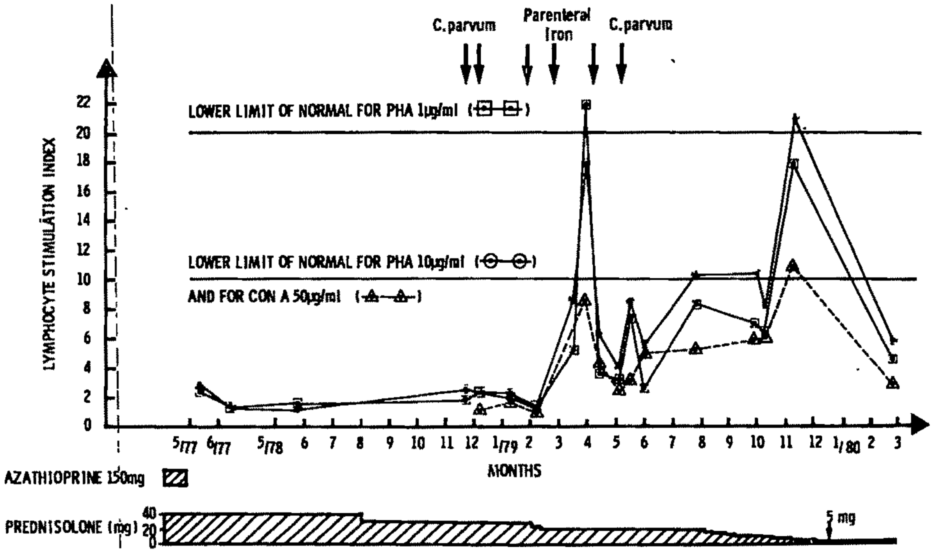

FIGURE 2. Lymphocyte responses to PHA and Con A expressed as stimulation index (ratio of counts/min stimulated to counts/min unstimulated). Counts from unstimulated cultures remained unchanged during parenteral iron treatment.

defective immune function, and thereby possibly to promote healing of the ulceration, he was treated with Coparvax (*Corynebacterium parvum* suspension, Burroughs Wellcome Ltd., 7 mg dry weight of formalin killed organisms per ml) 0·5 ml intravenously and 1·5 ml intramuscularly, followed 2 weeks later by a further 0·5 ml intravenously. *Corynebacterium parvum* has been reported to be a potent immunostimulant in experimental animals and preliminary clinical data in man have suggested that it may be useful for some immunologically compromised patients (Milas & Scott, 1978). This therapy, however, failed to produce any significant immunological or clinical change. A course of dapsone at 100 mg daily for 8 weeks (Soto, 1970) also failed to induce healing.

In view of an unexplained iron deficiency with failure to respond to oral iron, the patient received intravenous iron replacement therapy. Following this, the serum iron and stainable marrow iron stores returned to normal and the response of the patient's lymphocytes to PHA and Con A showed a striking but transient increase to the lower limits of normal (Fig. 2). The patient received prednisone 20 mg daily throughout this period. An injection of 0·5 ml Coparvax after the iron replacement had no further effect. Subsequent gradual reduction in the steroid dosage to a maintenance level of prednisone 5 mg daily has coincided with a period of healing of pyodermatous areas. Cutaneous anergy has persisted, however, and although there has been a modest improvement in immune function, *in vitro* lymphocyte responses have remained subnormal (Fig. 2).

DISCUSSION

The significance of immune dysfunction in the pathogenesis of pyoderma gangrenosum remains enigmatic. Several patients have been described without any demonstrable immune deficit (Lynch & Bergfeld, 1978; Holt *et al.*, 1980) whilst the diversity of immunological abnormalities found in other cases suggests that they may, at least in part, be epiphenomena. Alternatively, this diversity might reflect heterogeneity in the aetiology or pathogenesis of the disease. Altered leukocyte random migration or chemotaxis has occurred mainly in patients with underlying haematological disorders (reviewed by Norris *et al.*, 1978; Holt *et al.*, 1980). Leukocyte migration was normal in our patient, but neutrophil phagocytosis was reduced. Defective phagocytosis in pyoderma gangrenosum has been previously noted by Michäelsson *et al.* (1976), Beurey *et al.* (1977), Brandt *et al.* (1977), Norris *et al.* (1978) and Holt *et al.* (1980).

Cutaneous anergy, present in our patient, has been reported by many groups (Delescluse, De Bast and Achten, 1972; Lazarus *et al.*, 1972; Arala-Chaves, Proença & De Sousa, 1974; Stolman *et al.*, 1975; Michäelsson *et al.*, 1976; Clayton, Feiwel & Valdimarsson, 1977). Partial impairment of lymphocyte function with absent PHA-induced lymphocyte cytotoxicity (Delescluse *et al.*, 1972), dissociation between normal blastogenic response and absent macrophage inhibitory factor production (Lazarus *et al.*, 1972; Stolman *et al.*, 1975) or moderate reduction in lymphocyte transformation (Holt *et al.*, 1980) has been recorded chiefly in patients with an associated arthritis. However, two patients with idiopathic pyoderma gangrenosum and markedly impaired lymphocyte mitogen responses have been described (Arala-Chaves *et al.*, 1974; Brachtel & Lemmel, 1976). Clayton *et al.* (1977) reported a further patient with red cell aplasia, lymphopenia, a very low T-cell count, grossly impaired lymphocyte function and a serum factor which blocked the transformation of normal lymphocytes. Healing occurred despite persistent T-cell impairment. The immunological features in this case closely resembled those found in our patient. Serum factors which inhibit lymphocyte transformation have been described in a variety of other conditions, including tuberculosis, leprosy, syphilis, histoplasmosis, chronic hepatitis and cirrhosis (reviewed by Brattig & Berg, 1976) as well as in graft-versus-host disease (McMaster & Levy, 1975) and carcinomatosis (Glasgow *et al.*, 1974).

An association between candidiasis, iron deficiency and defective cell-mediated immunity, as in our case, is well recognized (Valdimarsson *et al.*, 1973). Iron deficiency may impair immune function and iron therapy has been reported to restore lymphocyte function (Fletcher *et al.*, 1975). Although parenteral iron replacement was followed by a temporary increase in lymphocyte reactivity towards normal, full restoration of immune function was not achieved in our patient.

Both our patient and the one reported by Clayton *et al.* (1977) were receiving corticosteroid therapy, and it is possible that this may have contributed to their impaired cell-mediated immunity. The effect of corticosteroid administration on lymphocyte proliferation is controversial (Parillo & Fauci, 1979), but Allison (1979) states that corticosteroid levels in excess of those likely to be achieved in therapy are required to inhibit lymphocyte mitogen responses. We have observed normal activation of lymphocyte thymidine incorporation in several patients receiving prednisone at up to 40 mg daily. Conversely, 3 patients with pyoderma gangrenosum who have never received corticosteroids have shown marked impairment of lymphocyte function *in vitro* (Valdimarsson, unpublished observations).

High dose corticosteroid therapy, with or without azathioprine, is usually effective, and despite serious side-effects remains the treatment of choice in patients with acute, rapidly progressive pyoderma gangrenosum (Holt *et al.*, 1980). A combination of high dose oral steroids and azathioprine eventually failed to control the disease in our patient and the corticosteroid therapy resulted in extensive vertebral collapse. Reduction in corticosteroid therapy has coincided with some improvement in lymphocyte function and a period of clinical resolution. Although this association may be fortuitous, other patients with markedly impaired lymphocyte function have failed to respond to corticosteroids (Clayton *et al.*, 1977) or azathioprine (Brachtel & Lemmel, 1976), and it has been suggested that immunosuppressive therapy may sometimes contribute to the development of pyoderma gangrenosum (Haim & Friedman-Birnbaum, 1976). Alternative forms of therapy, including immunostimulation (Arala-Chaves *et al.*, 1974; Brachtel & Lemmel, 1976) or plasmaphoresis (Clayton *et al.*, 1977) may therefore be advisable in patients with chronic idiopathic pyoderma gangrenosum and defective cell-mediated immunity, especially if a trial of steroid therapy has been unsuccessful.

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Porphyria cutanea tarda (PCT) in a patient with chronic renal failure on haemodialysis

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SUMMARY

Porphyria cutanea tarda, diagnosed by reduced levels of red cell uroporphyrin decarboxylase and raised plasma porphyrins, developed in a patient with chronic renal failure due to polycystic kidneys, treated with haemodialysis, who had normal total urinary and faecal porphyrins. Haemodialysis did not alter plasma porphyrin levels and we deduced that most of the plasma porphyrins were circulating in high molecular weight protein complexes.

Porphyria cutanea tarda (PCT) is a disease manifested by fragility, bullae, hyperpigmentation and hypertrichosis of the skin in sun exposed areas (Meyer & Schmid, 1978). Abnormalities in porphyrin metabolism result from reduced uroporphyrinogen decarboxylase activity (Kushner, Barbuto & Lee, 1976; Felsher & Kushner, 1975). Alcohol ingestion, iron overload and other exogenous agents have been implicated in provoking disease activity in the predisposed individual (Meyer & Schmid, 1978). A recent report has implicated chronic renal failure with haemodialysis as causative (Poh-Fitzpatrick *et al.*, 1978); but the diagnosis of PCT was not documented as it was in our patient by reduced uroporphyrinogen decarboxylase activity.

MATERIALS AND METHODS

Plasma and red cell porphyrins were determined by a fluorometric method on an Aminco-Bowman scanning spectrofluorometer using a coproporphyrin I standard (Sigma) and a fluorescence excitation of 405 nm and emission of 609 nm (Poh-Fitzpatrick *et al.*, 1974). The following tests were determined by Abbott-North-western Hospital Corporation (Dr C.J.Watson's Laboratory): urinary and faecal porphyrin, faecal isocoproporphrin, thin layer chromatography of plasma and erythrocyte porphyrin, and plasma δ-aminolaevulinic acid (ALA) and porphobilinogen (PBG).

Molecular sieve chromatography was performed with Ultragel 34 (LKB) in a 14 cm × 90 cm column and 15 mM NaCl, 50 mM Tris, pH 7·4 buffer, collecting 3 ml per tube, at a rate of 50 ml per h. Molecular sieve chromatography of plasma porphyrins was monitored by an Aminco-Bowman scanning spectrofluorometer.

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To determine if plasma porphyrin complexes were protein bound, plasma samples were digested with a mixture of bacterial protease (Sigma protease) at 37°C for 48 h.

Red cell uroporphyrinogen decarboxylase activity was performed by the method of Kushner *et al.* (1966).

Six patients with chronic renal failure and haemodialysis, one of whom also had a blistering disease resembling PCT, were the renal failure controls. Normals were selected from laboratory personnel.

CASE REPORT

A 51-year-old Caucasian woman presented in August, 1978 with a 5 month history of blisters and hypertrichosis of the face necessitating frequent shaving. She had a history of polycystic kidney disease and 20 years of chronic renal failure. Two renal transplants had been rejected. She was maintained on chronic haemodialysis twice weekly for 2 years prior to the development of her skin lesions. During the course of her haemodialysis she had been given injections of iron-dextran and was placed on anabolic steroids. Her mother had had polycystic kidney disease; but none of her family members ever had clinically apparent PCT. Medications included digoxin and nitroglycerin for congestive heart failure due to atherosclerotic heart disease. She did not drink alcohol and had never taken oestrogens. During her initial dermatological examination she had blisters on the face and depigmented scars on the dorsum of each hand. She had facial hyperpigmentation, most apparent under the eyes, and hypertrichosis of her eyebrows, chin and upper lip. Laboratory studies reflected chronic renal failure with a persistant normochromic, normocytic anaemia (haemoglobin 8 gm/dl, haematocrit 24%); and chronic uraemia with blood urea nitrogen over 100 mg/dl and creatinine over 15 mg/dl prior to dialysis. Liver function tests (serum glutamic oxalacetic transferase, lactic dehydrogenase and bilirubin) were within normal limits. Serum iron and iron binding capacity were repeatedly normal, while the serum ferritin level was elevated at 1916 μg/ml, both before and after phlebotomy (normal 0–18 μg/ml). Biopsy of a blister showed sub-epidermal bullae with festooning of the dermis. No dermal inflammatory infiltration or dermo-epidermal junction deposition of IgG, IgM or C3 by indirect immunofluorescence was detected.

After treatment with three 500 ml phlebotomies, clinical symptoms abated and the total porphyrin concentration in plasma decreased from 60·0 μ/dl to 14·3 μ/dl.

RESULTS OF PORPHYRIN STUDIES

The results of plasma, red cell, urine, faeces and dialysate porphyrin measurements are presented in Table 1. The plasma concentration of total porphyrins was increased to 60·6, 66·3, and 14·3 μg/100 ml on three occasions in our patient, while other renal failure and dialysis patients (2·0–6·0 μg/dl) and normal controls had much lower levels. Faecal isocoproporphyrins (Elder, 1972) were not detected while the patient was in clinical remission.

Plasma porphyrin levels and clinical symptoms were unchanged by haemodialysis with membranes with molecular filtration limits of 5,000 and 20,000 and no porphyrins were detected in the dialysate. To evaluate why porphyrins were not filtered by haemodialysis, plasma was separated by molecular sieve chromatography. Over 95% of the plasma porphyrins eluted in the void volume (molecular weight over 200,000) when the disease was active and porphyrin levels were 60 μg/100 ml (Fig. 1). After protease digestion, most of the porphyrins migrated in the total volume and there was an absence of the high molecular weight porphyrin complexes indicating that these complexes were protease digestible, and that the porphyrins were protein bound.

The red cell uroporphyrinogen decarboxylase activity was 0·21 nmol of 7, 6, 5 and 4—carboxyl porphyrinogens formed per hour per mg haemoglobin (normal 0·30–0·42).

TABLE I.

Specimen	Date	Porphyrin level		Normal value
Plasma	9/8/78	Total porphyrin	60·6 µg/dl	0–0·9 µg/dl (normal(s)
	14/9/78	Total porphyrin	66·3 µg/dl	2·0–6·0 µg/dl
	10/1/79	Total porphyrin	14·9 µg/dl	(renal failure—hemodialysis patient)
		Uroporphyrin	8·2 µg/dl	0
		Coproporphyrin	2·3 µg/dl	0
		Protoporphyrin	1·3 µg/dl	0
		ALA	10·8 µg/dl	0–10·8 µg/dl
		Porphobilinogen	none detected	0–4·4 µg/dl
Erythrocytes	11/8/78		49·0 µg/dl	2·2–92 µg/dl
Urine	10–11/8/78	Total porphyrin	3·2 µg/24 h	50–290 µg/24 h
Faeces	11/8/78	Coproporphyrin	1025·0 µg/24 h	400–1200 µg/24 h
		Uroporphyrin	182·0 µg/24 h	10–40 µg/24 h
		Protoporphyrin	642·0 µg/24 h	<1800 µg/24 h
	10/1/79	Isocoproporphyrin	none detected	
Dialysate	14/8/78		none detected	

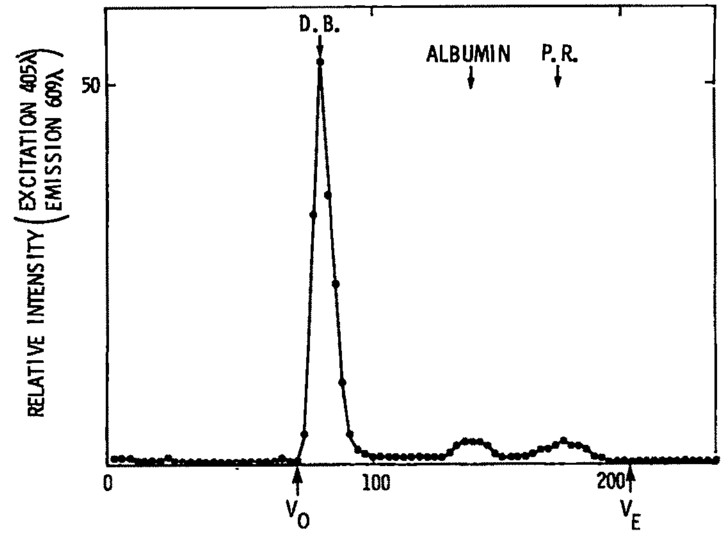

FIGURE 1. Molecular sieve chromatography of plasma, prior to phlebotomy, on Ultragel AcA 34. The column was calibrated with dextran blue (D.B.), albumin (Sigma), phenol red (P.R.), and coproporphyrin I (Sigma).

DISCUSSION

This paper presents a patient with chronic renal failure, treated by haemodialysis, who developed typical clinical and pathological features of porphyria cutanea tarda. The diagnosis was documented by reduced levels of red cell uroporphyrinogen decarboxylase activity, the enzyme that has been demonstrated to be reduced in the red cells and liver of patients with PCT (Kushner *et al.*, 1976; Felsher & Kushner, 1975).

Characteristically in PCT the diagnosis is ascertained from elevated urinary uroporphyrins, and plasma porphyrins are not necessary for diagnosis (Meyer & Schmid, 1978). However, in this patient, porphyrin metabolism was altered by her chronic renal failure, so that the total urinary porphyrin concentration was not elevated, but plasma total porphyrins and uroporphyrins were markedly elevated. Two patients previously reported with PCT and chronic renal failure, treated by haemodialysis, had similar biochemical findings (Poh-Fitzpatrick *et al.*, 1978), with urinary porphyrins only mildly elevated while plasma porphyrins were markedly so and haemodialysis failed to decrease the porphyrin levels. High molecular weight plasma porphyrin complexes were suspected as the reason but not demonstrated.

Our data indicate that plasma porphyrins were circulating in high molecular weight protein bound complexes since: (1) haemodialysis with a molecular weight exclusion below 20,000 failed to remove porphyrins (molecular weight below 1,000); (2) 95% of the plasma porphyrins present during her acute illness were present in the excluded volume on molecular sieve chromatography (molecular weight greater than 200,000); and (3) protease digestion eliminated most of the high molecular weight complexes and increased the amount of free porphyrin on molecular sieve chromatography. Binding of plasma porphyrin to high molecular weight protein may have further impeded renal filtration of porphyrins in this patient with severe renal disease.

In patients with chronic renal failure on haemodialysis a bullous dermatosis has been described that mimics the clinical and pathological features of PCT, but with normal urinary and faecal porphyrins (Gilchrest, Rowe & Mihm, 1975; Brivet *et al.*, 1978). Our patient presented with a bullous dermatosis associated with chronic renal failure and normal urinary and faecal total porphyrins. The diagnosis of PCT was documented by elevated plasma porphyrins and reduced red cell uroporphyrinogen decarboxylase. It is, therefore, recommended that all patients with bullous dermatoses and chronic renal failure be evaluated by plasma porphyrin levels, and, if possible, uroporphyrinogen decarboxylase activity in addition to urinary and faecal porphyrins.

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British Journal of Dermatology (1981) **104**, 579.

Porphyria cutanea tarda in a haemodialysed patient

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SUMMARY

A bullous dermatosis, that arose about 2 years after the beginning of haemodialysis treatment, was due to a genuine hereditary porphyria cutanea tarda (PCT). The plasma porphyrins were extraordinarily high. Neither the residual renal function nor the haemodialysis—using different techniques and different materials—succeeded in reducing the plasma porphyrin levels to that usually found in PCT. The serious and rapid evolution of the cutaneous lesions towards a scleroderma-like state might have been due to this high level of plasma porphyrins and to their passage into the tissues. The clearance of porphyrins is compared with that of 162 subjects affected by PCT. The porphyrin content in the plasma of seventy-five non-porphyric subjects undergoing maintenance dialysis was also studied.

B.C., a 63-year-old woman, who has been under dialysis treatment since 1976. In 1978 the typical cutaneous manifestations of PCT appeared and, in the course of a few months, sclerodermiform patches appeared very rapidly on the backs of the hands, on the face and on the scalp, with patches of cicatricial alopecia. Laboratory investigations were normal other than those for porphyrin metabolism:

Urine: total porphyrins 3·39 mg/l, 0·508 mg/24 h (residual diuresis 150 ml/24 h), with the typical pattern of PCT. δALA 0·0137 mg/24 h; porphobilinogen 0·0432 mg/24 h.

Plasma: total porphyrins 1·51 mg/l; δALA 0·065 mg%. Faecal and red cell porphyrins were normal. Investigation of the patient's relatives showed that a sister, a son and a niece were affected by PCT.

The average of several determinations of plasma porphyrins before and after haemodialysis and haemofiltration was 1·163 mg/l before and 1·118 mg/l after (the amount of porphyrins in the dialysis fluid ranged between 1 and 3 mg), with no correlation either with the use of various membranes with different cut-off (from 3000 to 50,000 daltons (M.W.) or with the different techniques used. On the contrary, plasma δALA showed a sharp decrease after haemodialysis. The clearance of porphyrins was 0·233 ml/min.

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The clearance of porphyrins, checked in a large control series (162 PCT patients without nephropathy) was 22·20±9·02 ml/min; this clearance was about 19% of the creatinine clearance determined simultaneously.

Increased plasma porphyrins were found in forty-six of seventy-five non-porphyric patients on haemodialysis for renal failure.

The sclerodermiform skin changes in PCT are well-known, but occur over several years and not a few months. The explanation of this course might lie in the unusually high levels of plasma porphyrins, with an increased passage into the tissues and an increase in the photodynamic activity. These high levels (about ten times the average of 261 PCT patients) are clearly due to renal failure: in fact, the clearance of porphyrins in our patient is about 100 times less than in other PCT patients. On the other hand, haemodialysis and haemofiltration appear to be unable to remove sufficient porphyrins because of their binding to proteins.

In these conditions, the plasma concentration of porphyrins reflects the hepatic production of porphyrins, and without possibility of adequate compensation. On the contrary, compounds such as δALA and PBG (as well as urea and creatinine)—with a lower molecular weight and *without* binding to plasma proteins—have been found to diffuse easily into the dialysis fluid. These findings correlate with our data that in about 60% of non-porphyric dialysed patients the plasma porphyrins are above normal.

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Cutaneous ultrastructural diagnosis of ceroid-lipofuscinosis

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SUMMARY

A skin biopsy from a 6-year-old girl was examined by electron microscopy and the diagnosis of ceroid-lipofuscinosis (CL) was confirmed. This has not been reported in the dermatology literature, because of the absence of specific skin manifestations. However, as we have shown, various cells of skin, including fibroblasts, Schwann cells, eccrine serous cells, endothelial cells and smooth muscle cells, accumulated characteristic substances to produce the finger print pattern (FPP) or the curvilinear profile (CLP) which are thought to be diagnostic for CL. Cutaneous ultrastructural research in such cases is important as asymptomatic skin disorders may exist in other congenital storage diseases.

Ceroid-lipofuscinosis (CL) is a storage disease which has been differentiated from amaurotic familial idiocy and is associated with the accumulation of ceroid or lipofuscin-like substances in various organs, especially in the nervous system (Zeman *et al.*, 1970). The major clinical signs of CL are visual disturbances, grand mal convulsions, gait disturbances and mental retardation and there is a progressive deterioration in the patient's condition.

Although no specific visible cutaneous manifestations have been reported in CL, the accumulation of characteristic substances in the skin has been reported (Carpenter, Karpaty & Andermann, 1972; Martin & Jacob, 1973).

Shown by electron microscopy, they have been described as the finger print pattern (FPP) and the curvilinear profile (CLP) and are thought to be diagnostic of CL. In recent years, the importance of extraneural electron microscopic diagnosis using skin, blood lymphocyte and urinary sediment has been emphasized (Martin & Jacob, 1973; Stekhoven & van Haelst, 1976; DeBaecque, 1975). Involvement of the skin in the late infantile type and in the juvenile type was first reported by Carpenter *et al.* (1972). Martin & Jacob (1973), Martin & de Groote (1974), O'Brien *et al.* (1975), Arsenio-Nunes & Goutieres (1975), Ceuterick *et al.* (1976) and Farrell & Sumi (1977) have reported

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on the skin involvement in various types of CL and stressed the importance of ultrastructural examination of punch biopsied skin as an easy and safe method for establishing the diagnosis. Nevertheless, in the field of dermatology we have found no published reports about CL, because of the absence of specific skin manifestations. In this study, the accumulation of FPP and CLP in different cells of the skin of a patient has been examined.

PATIENT AND METHODS

A girl aged 6 years had normal psychomotor development until the age of 2 years. The first symptom was loss of vision followed by nystagmus, myoclonus and later, gait disturbances.

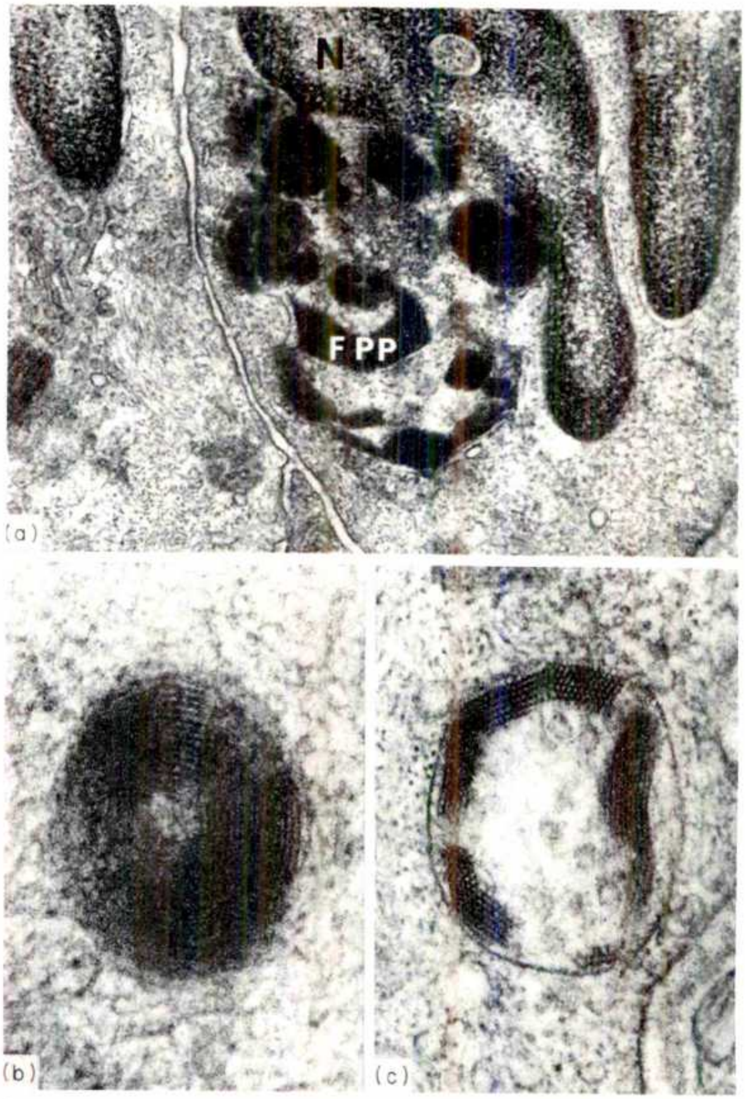

FIGURE 1. Cutaneous endothelial cell; (a) Showing many finger print patterns (FPP) in a lysosome. (N) nucleus (× 36,000). (b) Higher magnification of a typical lamellar FPP (× 163,000). (c) Higher magnification of a FPP showing lattice structures (× 117,000).

At the age of 4 years 5 months pigmentary degeneration of the retina was noted and mental deterioration and grand mal convulsions also developed. At the age of 6 years, a CT scan revealed slight brain atrophy, the EEG showed abnormal spikes, no response was elicited by electroretinography and various titrated lysosomal enzymes were within the normal ranges. No obvious skin manifestations have been seen in the course of this disease.

Tissue for electron microscopy was obtained from skin on the left forearm by punch biopsy and cut into smaller blocks which were fixed in 2% osmium tetroxide in 0·1 mol collidine buffer at pH 7·4 for 2 h and postfixed in unbuffered 6% glutaraldehyde for 2 h (Kanaseki & Kadota, 1969). The tissue was stained with 2% uranyl acetate before dehydration, and then embedded in Epon 812. Thin sections were stained with 2% uranyl acetate and Millonig's lead procedure and examined with the Hitachi HS-9 electron microscope.

RESULTS

The so-called finger print pattern (FPP) and curvilinear profile (CLP) were both found in various skin cells, i.e. endothelial cells (Fig. 1), smooth muscle cells (Fig. 2), eccrine serous cells (Fig. 3), Schwann cells and fibroblasts. In the basal epidermal cells, the accumulation of lipid-like substances was seen and the structural characteristics differed in the various cells.

Endothelial cells mainly showed an accumulation of FPP (Fig. 1) while smooth muscle cells and Schwann cells had dominant CLP (Fig. 2). Eccrine serous cells often showed the coexistence of FPP, CLP and lipid droplets in a circumscribed limiting membrane and no single type was dominant (Fig. 3). Fibroblasts had only atypical lamellar, non-packed, loosely coiled FPP type substances.

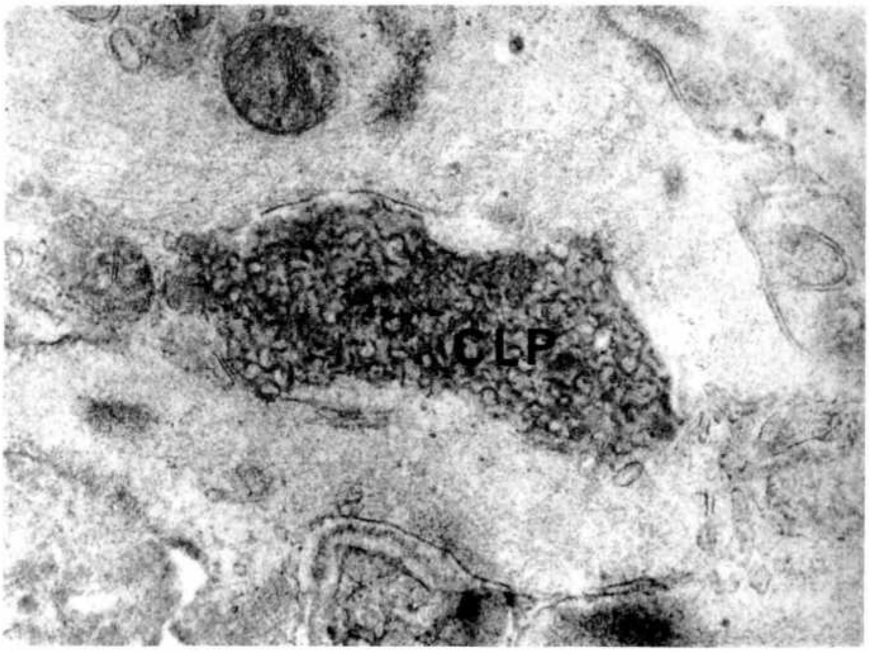

FIGURE 2. Curvilinear profile (CLP) in a smooth muscle cell (× 47,000).

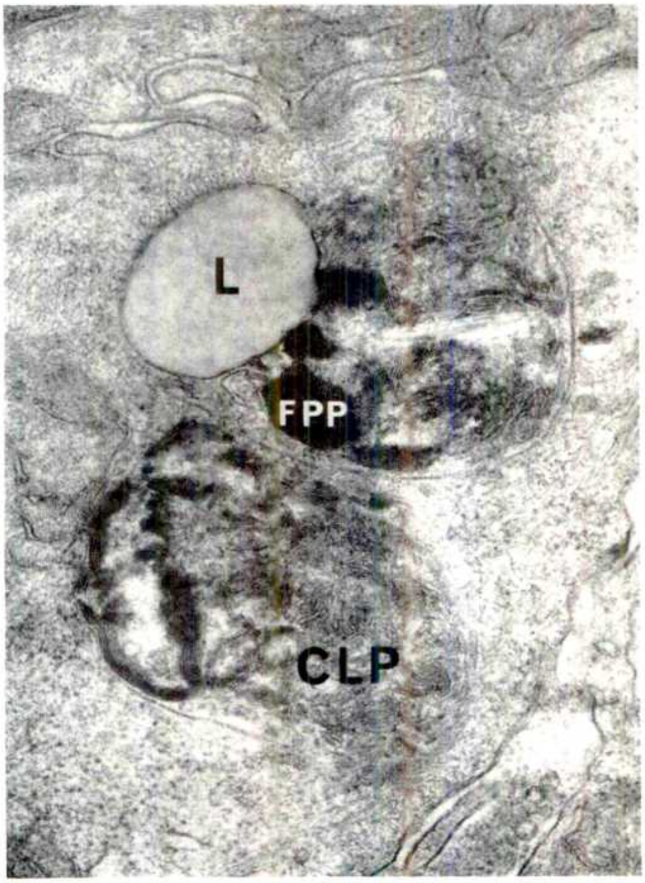

FIGURE 3. Eccrine serous cell, containing finger print pattern (FPP), curvilinear profile (CLP) and lipid droplet (L) in a circumscribed limiting membrane (×60,000).

COMMENT

Ceroid-lipofuscinosis resembles amaurotic familial idiocy with the exception of gangliosidosis such as Tay-Sachs disease. It is thought to be identical with Jansky-Bielschwsky disease, Vogt-Spielmeyer disease and Kufs disease. Zeman *et al.* (1970) suggested the name ceroid-lipofuscinosis from the ultrastructural appearance of accumulated substances. Although there have been many reports about the ultrastructure of accumulated substances in various organs, the first report of accumulation in the skin was made by Carpenter *et al.* in 1972. The significance of skin involvement and diagnostic value of ultrastructural examination in the late infantile, juvenile and Santavuori-Haltia types of CL have been studied by a growing number of authors. Recently, Martin & Ceuterick (1978) reported on eighteen cases of ceroid-lipofuscinosis examined by electron microscopy, and they also emphasized the diagnostic importance of skin biopsy.

In the skin of this 6-year-old girl, we found two types of characteristic accumulated substances (finger print pattern, FPP and curvilinear profile, CLP). CL was diagnosed and was classified as either the late infantile type from its onset accompanied by visual failure, or as the juvenile type from the age of onset. In both types, FPP and CLP have been reported and therefore the clinical classification cannot be determined only by the characteristics of the accumulated substances. Two types of substances have been shown in various skin cells and in this case accumulation was easier to find in eccrine serous cells and endothelial cells than in other cells. In Schwann cells, smooth muscle cells and fibroblasts, accumulation was rarely observed, but lamellar non-packed loosely coiled FPP in the fibroblasts were characteristic.

Some symptomatic visible skin manifestations in CL, i.e. acrocyanosis, seborrhoeic dermatitis (Sjögren, 1931), hyperpigmentation, poliosis and hirsutism (Zeman *et al.*, 1970) have been reported. As these are found in other degenerative nervous diseases and may result from the use of anticonvulsant drugs they cannot be regarded as being specific for CL.

This may explain the absence of reports in the dermatological literature. Recently, Martin & Ceuterick (1978) have discussed the diagnostic value of ultrastructural studies using skin biopsy specimens in some congenital storage diseases which had no specific symptomatic skin involvement.

We think that the asymptomatic skin disorders found in these congenital storage diseases should be regarded as dermatological diseases despite the absence of visible skin manifestations.

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Cerebriform tongue—a clinical sign in pemphigus vegetans

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SUMMARY

Ten cases of pemphigus vegetans of Neumann type are reported. Six of these showed clinically distinctive changes in the tongue for which the term cerebriform tongue is proposed. Histological examination of the tongue showed typical features of pemphigus vegetans.

Pemphigus vegetans is a variant of pemphigus vulgaris occurring in patients having an increased resistance to their disease (Lever & Lever, 1975). The diagnosis is easily made based on the clinical and histopathological features. This variant is of two types—the malignant Neumann type and the benign Hallopeau type. In pemphigus vegetans of Neumann type the vermillion border of the lip is frequently affected and deep fissures may form between the vegetations. In the Hallopeau type the vermillion border of the lips also frequently shows papillomatous hyperplasia (Lever, 1953). To our knowledge, similar papillomatous hyperplasia on the dorsum of the tongue has not been reported in the literature.

MATERIALS AND METHODS

Cases studied attended our Skin Clinic during the years 1968–1979. Out of 108 cases of pemphigus there were twelve cases of pemphigus vegetans. Cerebriform tongue was observed in 50% of cases of pemphigus vegetans. Tzanck smears for acantholytic cells were taken in patients with pemphigus vegetans who had bullous lesions on the skin. Biopsy was performed from the vegetative plaques and from the cerebriform tongue and the sections stained with haematoxylin and eosin. The clinical features of the twelve cases of pemphigus vegetans are summarized in Table 1.

RESULTS

Of the twelve cases of pemphigus vegetans three were male and nine female. The age range was between 4 and 60 years. Ten cases were of Neumann type and two of the female cases were of Hallo-

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TABLE 1. Summary of the clinical features of twelve cases of pemphigus vegetans

Case no. and type	Age in years	Sex	Type of skin lesions	Sites of distribution	Duration of illness on admission	Mucous membrane involvement	Onset of mucous membrane lesion
Case 1 (Neumann type)	45	F	Vesicles, flaccid bullae and vegetative plaques	Axillae, chest, abdomen and groin	3 years	Cerebriform tongue	6 months after the onset of skin lesions
Case 2 (Neumann type)	42	F	Flaccid bullae and vegetative plaques	Scalp	2 months	Cerebriform tongue and ulcerated buccal mucosa	1 month after the skin lesions
Case 3 (Neumann type)	50	F	Flaccid bullae and vegetative plaques	Scalp and axillae	2 years	Cerebriform tongue. Ulceration in buccal mucosa and anogenital region. Oral candidiasis	Simultaneous occurrence with the skin lesions
Case 4 (Neumann type)	38	F	Vesicles and vegetative plaques	Axillae and groin	1½ years	Cerebriform tongue. Ulcerated buccal mucosa. oral candidiasis	6 months prior to the onset of skin lesions
Case 5 (Neumann type)	45	F	Flaccid bullae and vegetative plaques	Axillae, groin and vulva	10 days	Cerebriform tongue. Vegetative lesions in the vulval mucosa	Few years prior to the onset of skin lesions
Case 6 (Neumann type)	36	F	Vegetative plaques	Nape of the neck and labia majora	6 months	Cerebriform tongue and fissured lips. Oral candidiasis	2½ years prior to the onset of skin lesions

Case 7 (Newmann type)	36	F	Vegetative plaques	Neck	6 months	Vegetative lesions in the vulval mucosa. Candidiasis-vulvo-vaginitis	Few months prior to the onset of skin lesions
Case 8 (Hallopeau type)	12	F	Pustules, vegetative and crusted plaques	Neck, axillae and groin	4 months	Fissured lips	Few months prior to the onset of skin lesions
Case 9 (Hallopeau type)	4	F	Pustules, vegetative and crusted plaques	Scalp—'Cutis frontalis parietalis and verticis gyrata', neck and right cubital fossa	1½ months	No mucous membrane involvement	—
Case 10 (Neumann type)	40	M	Flaccid bullae and vegetative plaques	Axillae, chest and groin	3 months	Fissured tongue	1 month after the onset of skin lesions
Case 11 (Neumann type)	46	M	Vesicles and vegetative plaques	Scalp—'cutis parietalis and verticis gyrata', ears, axillae, chest, forearms and groin	4½ months	No mucous membrane involvement	—
Case 12 (Neumann type)	60	M	Vesicles vegetative and crusted plaques	Neck, axillae, groin and legs	6 years	Ulcerated buccal mucosa	Simultaneous occurrence with the skin lesions

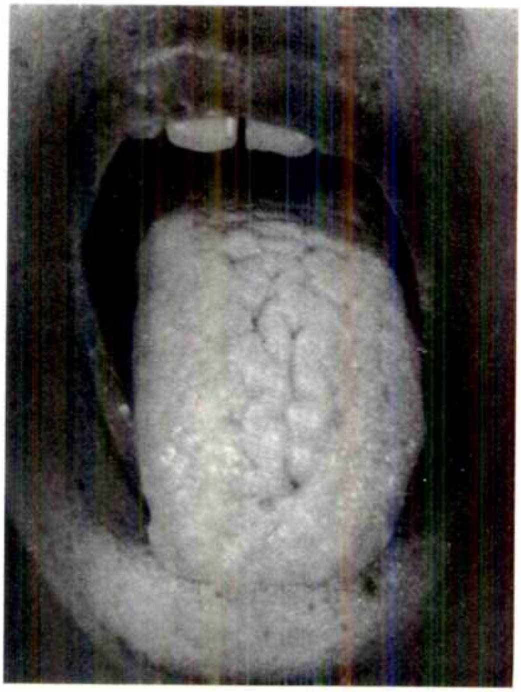

FIGURE 1. (Case 1) Pattern of sulci and gyri of cerebriform tongue.

peau type. Six cases of Neumann type showed cerebriform tongue (Fig. 1). One case of Neumann type and one case of Hallopeau type presented with fissured lips. One case of Neumann type showed a fissured tongue rather than a cerebriform tongue. One male case of Neumann type and one female case of Hallopeau type showed 'cutis verticis gyrata' of the scalp and skin biopsy of the scalp lesions showed the features of pemphigus vegetans. Eight cases had vesicles and bullae on the skin initially and all cases ultimately developed the characteristic vegetative plaques. Two cases had pustular lesions initially and these were of Hallopeau type. Tzanck smears did not show acantholytic cells in these cases. Hyperkeratosis, acanthosis, suprabasal lacunae, eosinophilic microabscesses within the rete ridges and chronic inflammatory cells in the dermis were observed in the biopsies from these two cases. Tzanck smears showed typical acantholytic cells in six of the eight cases of Neumann type who presented with bullous lesions on the skin on admission. Skin biopsy of the vegetative plaques in six cases of Neumann type showed typical features of pemphigus vegetans. Biopsy of the fissured lip in one case of Hallopeau type and of the cerebriform tongue in four cases of Neumann type also showed the features of pemphigus vegetans. Hyperkeratosis, acanthosis, suprabasal lacunae containing the acantholytic cells and eosinophils, villi lined by a single layer of basal cells projecting into the bulla, dilated blood vessels and chronic inflammatory cells in the dermis were observed in the biopsies of the cerebriform tongue.

DISCUSSION

Scrotal tongue or fissured tongue (lingua plicata) is dominantly inherited or acquired. Acquired scrotal tongue can occur in gout, syphilis, cerebriform naevus and lymphangioma. This entity is clinically characterized by a central longitudinal groove with shorter groves extending bilaterally like 'veins of a leaf'. In more severe cases the tongue may be lobulated (Butterworth & Strean, 1962a). Scrotal tongue can also occur in pernicious anaemia and Melkersson–Rosenthal syndrome (Pindborg, 1972).

The cases reported here showed a pattern of sulci and gyri over the dorsum of the tongue giving a 'cerebriform appearance', the morphological features of which differed from scrotal tongue. Cerebriform tongue may precede, coincide with or follow the skin lesions of pemphigus vegetans. Follow-up of these cases showed slow regression of the tongue lesions and a poor response to systemic corticosteroids.

Pemphigus vegetans has already been mentioned as one of the causes of secondary cutis verticis gyrata (Butterworth & Strean, 1962b). Two of our cases also showed such involvement of the scalp.

We believe that the papillomatous hyperplasia present in this disease may be the basis for both the 'cerebriform scalp' and 'cerebriform tongue'. In all cases of pemphigus the dorsum of the tongue should be observed for a cerebriform appearance, which if present may be an important diagnostic clinical sign of pemphigus vegetans since it may even precede the skin lesions by months or sometimes years.

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Cerebellar ataxia and sensory neuropathy associated with dermatitis herpetiformis

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SUMMARY

A patient with longstanding dermatitis herpetiformis developed cerebellar ataxia and sensory neuropathy. These neurological changes typical of coeliac disease have not previously been noted with dermatitis herpetiformis in the absence of coeliac disease. The possible pathogenesis is discussed.

Cerebellar ataxia and sensory neuropathy are recognized neurological complications of coeliac disease (Cooke & Smith, 1966). Dermatitis herpetiformis and coeliac disease not infrequently co-exist, dermatitis herpetiformis sometimes responds to a gluten free diet, and both diseases have a high incidence of HLA B8 (Cooke, 1976). Despite the considerable overlap between coeliac disease and dermatitis herpetiformis, neurological changes typical of coeliac disease have not been recorded in association with dermatitis herpetiformis. We report such a case.

CASE REPORT

A 62-year-old man presented in April 1977 with unsteadiness and weakness of both legs which had progressed in the previous 18 months. Dermatitis herpetiformis had been diagnosed in 1950 and treatment was commenced with dapsone, 100 mg daily. The dose was reduced to 50 mg daily in 1951 and maintained ever since. Several attempts at discontinuing dapsone had led to exacerbations of dermatitis herpetiformis. In 1956 a partial gastrectomy had been performed for duodenal ulceration.

Neurological examination revealed hypotonia and gross ataxia in the legs, but co-ordination in the arms and power in all limbs were normal. The biceps and triceps tendon jerks were present but the supinator, knee and ankle jerks were unobtainable. The plantar responses were equivocal. The gait was grossly ataxic and the patient walked with a stick. Touch, pin-prick and cold sensations were decreased symmetrically in both legs distal to mid-calf level and in both hands distal to the proximal

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interphalangeal joints. Vibration sense was absent in the lower limbs below the pelvis. Proprioception was normal and Romberg's sign negative. Intellectual function and the remainder of the physical examination were normal.

Electrophysiological studies in September, 1977 and June, 1978 showed normal motor nerve conduction velocities except in the right lateral popliteal nerve where conduction was slightly delayed. Sensory evoked potentials were not recorded from the sural and medial plantar nerves. The visual evoked responses were normal. The cerebrospinal fluid (CSF) was normal, and calculation of CSF/serum IgG clearance did not suggest intrathecal IgG synthesis. Computerized axial tomography revealed some atrophy in the Sylvian fissures, but the cerebellum appeared normal.

Skin biopsy from apparently normal skin showed IgA immunofluorescence at the basement membrane typical of dermatitis herpetiformis. Jejunal biopsy showed normal mucosal architecture.. The tissue type was HLA-A1, B8. Tests of malabsorption (serum vitamin vitamin B_{12}, red cell folate, Schilling test, faecal fat excretion) were normal. Other normal tests included a full blood count, protein electrophoresis, blood sugar, serology for syphilis, and radiographs of the chest and skull.

Follow up over 3 years has shown some progression of both ataxia and neuropathy. Initially this was thought to be due to dapsone neuropathy, though the presence of ataxia was not characteristic. Cessation of dapsone on many occasions has resulted in marked worsening of dermatitis herpetiformis, despite sulphapyridine as alternative therapy, but no improvement in the neurological state.

DISCUSSION

Our patient with dermatitis herpetiformis demonstrates neurological changes typical of coeliac disease, but without histological or biochemical evidence of the latter. Dapsone is unlikely to be responsible because dapsone-associated neuropathy is predominantly motor or mixed (Gutman, Martin & Welton, 1976), and neither pure sensory neuropathy nor ataxia have been associated with this drug. Dapsone neuropathy usually occurs after a short duration of therapy, but in a single case it may have taken 18 years to develop (du Vivier & Fowler, 1974). Although our patient was on dapsone for 27 years, the dose was only one-quarter to one-sixth that of the case reported by du Vivier and Fowler (1974). Thus the smaller dose and longer duration of dapsone therapy would argue against this being drug related neuropathy.

Cerebellar ataxia and neuropathy are features of multiple sclerosis, but this is unlikely in the presence of normal visual evoked response, normal motor power, and normal cerebrospinal fluid IgG, the latter indicating absence of intrathecal IgG synthesis.

The aetiology of neuropathy in coeliac disease remains unknown though vitamin deficiences do not seem to be implicated (Cooke, 1976). The cerebellar syndrome may progress despite a gluten free diet, vitamin supplements and prednisone therapy (Finelli *et al.*, 1980). Fortunately our patient has deteriorated only slightly in the last 3 years.

On the basis of a single case it would be unwise to propose a mechanism for the presence of neurological changes typical of coeliac disease in a patient with dermatitis herpetiformis alone. However, this case suggests there may be a spectrum of disease—possibly linked to HLA B8—which may have resulted in this patient in skin and nerve lesions without the intestinal pathology. A search for subclinical neuropathy in a group of patients with dermatitis herpetiformis not on dapsone may be fruitful.

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Brief communications

Unsuccessful treatment of alopecia areata with dapsone

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Alopecia areata is associated with auto-immune endocrine diseases (Cunliffe *et al.*, 1969; Main *et al.*, 1975) and there has been speculation that auto-immune mechanisms are causally involved in its pathogenesis (du Vivier & Munro, 1975). Di-aminodiphenyl sulphone (dapsone) has been used with increasing frequency in diseases in which immunologically mediated inflammatory mechanisms have been implicated. Tofharn, Gernand & Hundeiker (1976) reported that dapsone used in combination with low dose prednisolone (8–12 mg) induced regrowth of hair in nine of eleven patients with alopecia areata. It would clearly be desirable to avoid the systemic use of corticosteroids completely. Therefore, the present study was performed to see whether dapsone alone would induce regrowth of hair in alopecia areata.

TABLE 1. Dapsone in alopecia areata

Patients	Age ± s.d.	Number with total alopecia	Duration of hair loss (years)	Duration of treatment (months)
Female n = 14	38·9 ± 19	7	9·1 ± 8·3 range 9 months–25 years	5 ± 4
Male n = 8	25·8 ± 9	6	3·3 ± 3 range 1–38 years	3·6 ± 1·6

Twenty-two patients whose alopecia had not responded to local triamcinolone injection (Table 1) received dapsone at an initial dose of 150 mg daily for 14 days. The dose was reduced to 100 mg daily for a further 10 weeks after which the extent of regrowth was assessed. Of fourteen females, two, with the diffuse form of alopecia areata, showed transient thickening of hair. In one, hair fell out while treatment continued, and in the other, hair fell out again shortly after cessation of therapy. Of eight males, three showed regrowth of a few hairs. One female and one male with complete hair loss showed partial regrowth of eyebrows and lashes.

Eight patients (seven female, one male) experienced side-effects in the form of headache, nausea and a general sense of malaise and tiredness. The side-effects were so severe that five females and one male gave up before they had completed 3 months treatment.

It is perhaps not surprising that dapsone should exert little if any effect in this condition in view of the increasing awareness that its actions are upon inflammation mediated by polymorphs rather than lymphocytes.

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Juvenile plantar dermatosis in an adult

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This recently described, self-limited dermatosis affects the feet of predominantly school-age children. It has rarely been reported in adults (Silvers & Glickman, 1968; Enta, 1972). The clinical appearance is highly suggestive: the distal half of the plantar surface is anhidrotic, glazed and fissured with sparing of the web spaces (Mackie & Husain, 1976). Occlusive footwear is believed to be the main cause. Shrank (1979) has shown that the feet become supersaturated with sweat, leading to oedema of the epidermis and obstruction of the sweat ducts. Allergic contact dermatitis is not a factor in the pathogenesis but several authors have found a high incidence of atopy among their patients or close relatives (Jepsen, 1979; Millard & Gould, 1977; Verbov, 1979).

We wish to report an adult with this dermatosis.

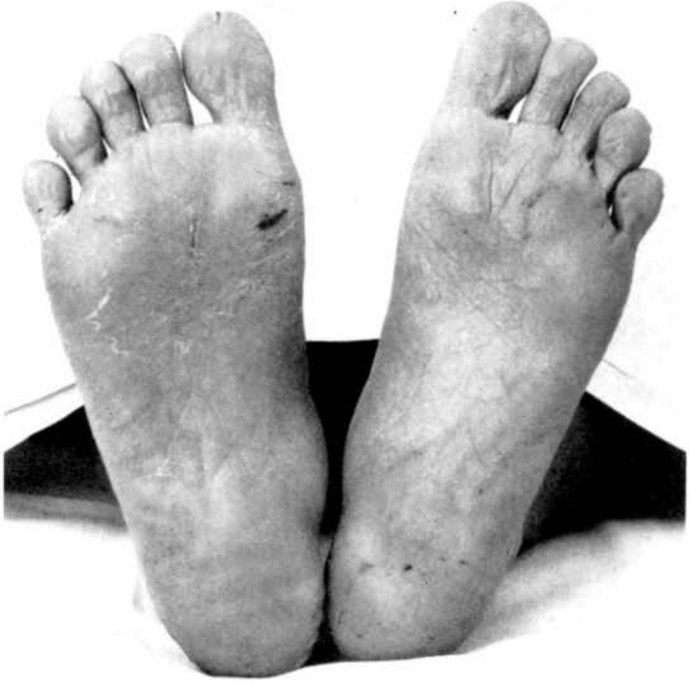

FIGURE 1. Symmetrical smooth glazed appearance over plantar aspect of distal feet. Note peripheral fissuring and scaling.

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CASE REPORT

A 23-year-old male Vietnamese refugee was admitted into Australia in January, 1980. In Vietnam he had not suffered from any skin disease nor from any atopic disorder nor was there any family history of similar diseases. With the onset of the Australian autumn he began wearing shoes regularly for the first time in his life. He soon developed a burning, pruritic dermatosis of both soles which did not respond to topical fluorinated steroid ointment. The distal half of the sole was glazed, erythematous and scaly with painful fissures along the skin creases. The toe spaces were spared. Starch iodine testing showed that the affected areas were anhidrotic (see Fig. 1). Skin scrapings for fungal culture were taken on two occasions but no fungus was isolated. There was no evidence of any other skin disease.

He showed rapid improvement when he began to wear cotton socks and leather sandals instead of synthetic socks and shoes.

DISCUSSION

This adult developed a disorder indistinguishable from juvenile plantar dermatosis when he began to wear shoes regularly for the first time.

We believe that occlusive footwear was the prime aetiological factor in the development of this condition and that with greater awareness it may be recognized more frequently in adults.

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Comment

The current status of histamine receptors in human skin: therapeutic implications

There has been a significant increase in our knowledge and understanding of cutaneous histamine receptors during the last five years. Moreover it is now appreciated that histamine is involved in a much wider spectrum of physiological and pathological events within the skin than was previously thought. There is increasing evidence implicating histamine in the pathogenesis of the skin lesions due to circulating immune complexes (Knicker & Cochrane, 1968). Local release of histamine by facilitating the sub-endothelial penetration of immune complexes would allow the latter to exert maximal vascular damage through complement activation and granulocyte infiltration. Histamine may also have a regulatory role in delayed hypersensitivity skin reactions, including contact allergic dermatitis. Histamine mediated modulation of T-lymphocyte function via cell surface receptors may be one important mechanism (Rocklin, 1976). Histamine may also exert a regulatory effect on epidermal growth (Iizuka *et al.*, 1976).

This review examines recent work on vascular, itch, immunological and epidermal receptors in human skin. The antiandrogen effect of H_2 receptor antagonists is also discussed.

VASCULAR RECEPTORS

Following the preliminary observations of Wyllie, Hesselbo & Black (1972) and Burland *et al.* (1975), and the identification of two synthetic histamine analogues, 2-methyl histamine and 4-methyl histamine, with predominantly H_1 and H_2 receptor agonist activity respectively (Black *et al.*, 1972), Robertson & Greaves (1978) have provided further persuasive evidence for the existence of both types of histamine receptor in human skin blood vessels. They showed that both 2-methyl histamine and 4-methyl histamine evoked dose-related erythema. However, 2-methyl histamine, but not 4-methyl histamine, caused erythema mediated by an axon reflex, suggesting that the axon reflex is due to an H_1 action of histamine. In contrast, the direct vasodilator action of histamine involves both H_1 and H_2 histamine receptors. Chlorpheniramine (an H_1 antagonist) but not cimetidine (an H_2 antagonist) inhibited the erythema due to 2-methyl histamine while both antagonists inhibited the erythema due to 4-methyl histamine. Neither antagonist significantly modified wealing due to either histamine analogue.

Having adduced evidence for the presence of both types of histamine receptor in human skin blood vessels, Marks & Greaves (1977) studied the effects of chlorpheniramine and cimetidine, alone and in combination, on erythema and wealing induced by intradermal histamine and compound 48/80. Administration of both drugs together caused significantly greater suppression of histamine and compound 48/80 erythema than either drug alone. However, the combination did not produce significantly greater reduction of wealing than either drug individually.

The logical extension of these experimental studies was to assess the effect of an H_2 antagonist alone and in combination with an H_1 antagonist in various types of urticaria. Hitherto used antihistamines had predominantly H_1 blocking activity and an appreciable failure rate in the treatment

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of urticaria is widely acknowledged. Commens & Greaves (1978) in a randomized double blind study of nineteen patients with chronic idiopathic urticaria failed to show any significant therapeutic benefit (as assessed by reduction of the daily number of weals) following combination treatment with cimetidine and chlorpheniramine compared with either drug given alone or placebo. In contrast, in a study of ten patients with symptomatic dermographism, Matthews *et al.* (1979) demonstrated that chlorpheniramine and cimetidine together gave a greater reduction in the weal and flare response to graded dermographic stimuli than chlorpheniramine alone. These findings were extended by Kaur, Greaves & Eftekhari (1981) who, in a double blind, randomized cross-over trial of chlorpheniramine and cimetidine alone and in combination in twenty patients with dermographism, showed that the combination of chlorpheniramine and cimetidine was the only treatment that significantly reduced weal size, flare size and duration of weal, compared with placebo, although other treatments approached statistical significance. Furthermore, when the most effective of the four treatments was continued for a further 3 months without breaking the randomization code, more evidence of the superiority of combined chlorpheniramine and cimetidine was seen.

Also at the clinical level, Tan, Gaylarde and Sarkany (1979) have reported a synergistic effect of H_1 and H_2 antagonists in blocking alcohol-induced flushing. Roberts, Marney & Oates (1979) have demonstrated a similar synergistic effect in abolishing carcinoid flushing in a patient with metastatic gastric carcinoid syndrome.

Clearly, studies need to be performed in other conditions where histamine is known to be a mediator and the clinical efficacy of newer more potent histamine-H_2 receptor antagonists needs to be evaluated (Daley & Stables, 1980).

ITCH RECEPTORS

The central role of histamine as a mediator of pruritus is well established. Experimentally, histamine is known to cause pain when injected intradermally, and itching when applied intra-epidermally (Keele & Armstrong, 1964). The epidermal scarification technique of Greaves & McDonald-Gibson (1973) has provided a useful means of directly studying the pruritogenic properties of histamine and its synthetic analogues. Using this technique, Davies & Greaves (1980) showed that itching could be evoked by the receptor agonist, 2-methyl histamine. They also found that the itch thresholds for 2-methyl histamine were consistently much higher than for histamine ($P<0·001$), and that prior administration of chlorpheniramine raised the itch thresholds to both 2-methyl histamine and histamine itself significantly ($P<0·001$). In contrast, pruritus was not obtained with either of two H_2 agonists, 4-methyl histamine and dimaprit. Also, somewhat surprisingly, no evidence of synergism between 2-methyl histamine and either 4-methyl histamine or dimaprit was found. These results suggested that histamine-induced pruritus is mediated in part through the H_1 receptor and in part via an additional (but probably non-H_2) mechanism. That H_2 receptors play little or no part in histamine-induced itching receives further support from the studies of Hägermark, Strandberg & Grönneburg (1979). There is now some evidence for the existence of a further sub-class of histamine receptor (Eyre & Chand, 1979). The presence in the skin of a new sub-class of receptor mediating itch but not stimulated by either dimaprit or 4-methyl histamine remains an intriguing, but as yet unconfirmed, possible explanation. An alternative possibility that histamine is in part pruritogenic via an indirect pathway involving an oligopeptide, prostaglandin or other intermediate remains to be excluded. It is interesting to note that cimetidine administration has been reported to increase itching in patients with dermographism (Matthews *et al.*, 1979; Boss & Warin, personal communication) and in a patient with cold urticaria (Highet & Titterington, 1979). This may be due in part to an inhibitory effect on histamine N-methyl transferase by cimetidine.

As a proven H_2 antagonist, cimetidine has been used clinically in the treatment of the pruritus associated with a variety of systemic diseases. Thus there are now several reports of its use in the myeloproliferative diseases, especially polycythaemia rubra vera (Easton & Galbraith, 1978); Hess, 1979; Scott & Horton, 1979). Initial results were encouraging, but the last group of workers in a formal trial found cimetidine alone to be ineffective in controlling the pruritus. The drug has also been used on a much more empirical basis in the treatment of pruritus associated with cholestasis (Harrison *et al.*, 1979) and uraemia (Zappacosta & Hauss, 1979). In neither trial was cimetidine found to be effective although a preliminary report in patients with pruritus and Hodgkin's disease is more encouraging (Aymard *et al.*, 1980).

Cimetidine has also been used alone and in combination with an H_1 antihistamine in the treatment of patients with atopic eczema (Foulds & Mackie, to be published). Using a linear itch scale as an index of pruritus and clinical and photographic visual assessment, they found that cimetidine did not confer any therapeutic advantage when added to an H_1 antagonist.

IMMUNOLOGICAL RECEPTORS

Further possible evidence for the importance of histamine H_2 receptors in the pathophysiology of skin comes from the work of Avella *et al.* (1978) who tested cutaneous delayed hypersensitivity to four antigens before and after 6 weeks treatment with cimetidine. They showed that such therapy was associated with a significant increase in both erythema and induration and suggested that the augmentation of delayed hypersensitivity might be due to a blockade of feedback inhibition on regulatory T-lymphocytes. There are at least two alternative explanations (Greaves, 1978); firstly, that cimetidine would partly inhibit the immediate histamine induced reaction to antigen, leading to reduced local clearance of the latter and an augmented delayed hypersensitivity response; secondly, since cimetidine has been shown to inhibit the histamine-degrading enzyme N-methyl transferase (Corbett & Greaves, 1979), an augmented response might be due to reduced decay of histamine activity. Whatever the explanation of the observed enhancement of cutaneous delayed hypersensitivity, there is voluminous evidence to show that cimetidine enhances T cell function. This has been put to therapeutic use by Jorizzo *et al.* (1980) who successfully used cimetidine to enhance cell mediated immunity in patients with chronic mucocutaneous candidiasis.

The exact role of histamine as a modulator of cellular immune responses awaits to be elucidated, but there is increasing evidence of an important action via the activation of a T cell suppressor subpopulation. Shearer, Melmon & Weinstein (1972) first reported that T cells with suppressor function could be retained by an insolubilized histamine column. This suggested that some suppressor cells carried cell surface histamine receptors. Subsequently, histamine has been shown to inhibit lymphocyte-mediated cytotoxicity (Plaut *et al.*, 1973), the production of antigen induced migration inhibitory factor (MIF) (Rocklin, 1977) and lymphocyte proliferation (Wang & Zweiman, 1978). Hébert *et al.* (1980) have shown that histamine-incubated peripheral human blood lymphocytes lower the proliferative response to mitogens (phytohaemagglutinin and concanavalin A) and antigens (mixed lymphocyte culture). They also confirmed that, as with previous studies, the histamine effect is mediated through the H_2 receptor.

EPIDERMAL RECEPTORS

Epidermis is a major source of the cytoplasmic histamine degrading enzyme histamine-N-methyl transferase in human skin (Francis, Greaves & Yamamoto, 1977). This and other evidence implies an important role for histamine in epidermal growth and differentiation. Indirect evidence for the presence of histamine (H_2) receptors on epidermal cells comes from the work of Iizuka *et al.* (1976) using pig skin. In common with adrenaline, histamine is now known to activate epidermal cell adenyl cyclase activity, leading to a rapid increase in intracellular cyclic AMP concentration. More recently Taylor, Halprin & Levine (1980), using an *in vitro* system, have found that histamine may increase tritiated thymidine incorporation into epidermal DNA. However, considerable doubt exists that a histamine-induced epidermopoietic response is mediated by cyclic AMP since in the same paper Taylor *et al.* (1980) could not demonstrate that agents which elevate cyclic AMP concentration, including cyclic AMP itself, had any consistent effect on the 'S' phase of DNA synthesis. Possibly, histamine acts on the epidermis partly by inducing local prostaglandin formation since, at least in some tissues, histamine is known to evoke prostaglandin synthesis (Blackwell *et al.*, 1978). The concept that dermally derived histamine might exert a regulatory effect on epidermopoiesis and epidermal differentiation is exciting, since such a mechanism might in part explain the acanthosis and lichenification seen in atopic and other forms of chronic eczema.

THE ANTIANDROGEN EFFECT OF HISTAMINE H_2 ANTAGONISTS

Cimetidine has recently been found to have antiandrogen activity (Funder & Mercer, 1979; Winters, Bank & Loriaux, 1979; Van Thiel *et al.*, 1979). Although the exact mechanisms have not yet been fully elucidated, Sultan *et al.* (1980) have shown that cimetidine competes for dihydrotestosterone cytoplasmic binding sites in human genital skin fibroblasts.

Lyons, Cook & Shuster (1979) found partial inhibition of sebum excretion in acne patients treated with cimetidine. Further studies (Lyons & Shuster, 1980) showed that ranitidine, a related H_2 antagonist, also decreased sebum excretion. With both drugs slight improvement was seen in the acne of about half the patients. In contrast, Jones *et al.* (1980), in a double blind trial of cimetidine in thirty-nine patients with acne, were unable to confirm a reduction in sebum excretion or any clinical benefit.

In a preliminary report, Vigersky *et al.* (1980) demonstrated a decreased rate of hair growth in androgen-dependent hirsute women when treated with cimetidine.

In spite of the antiandrogenic activity of H_2 antagonists, the physiopathological relevance, if any, of histamine to androgen activity is currently unclear.

In conclusion, although rapidly expanding knowledge of the nature of histamine actions in skin is already providing a stimulus for clinical research, detailed studies of histamine at a molecular level, including studies of high affinity binding of histamine in skin, are badly needed before the role of histamine can be fully clarified.

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British Journal of Dermatology (1981) **104**, 607.

Correspondence

Patch test concentrations of grotan BK

SIR, Grotan BK (GBK) is a 79% aqueous solution of hexahydro-1,3,5 tris (2-hydroxyethyl)-s-triazine. It is used in metal working industries as a bacteriocide which is added to oil-in-water emulsions (cooling oils) in a final concentration of 0·15% by volume. Its role as a contact sensitizer has been reviewed by Rycroft (1978). Using concentrations for patch testing of 0·1–1·0%, different German investigators have found sensitization rates in exposed workers of zero (Schneider *et al.*, 1965), 1·8% (Düngemann, Borelli & Reber, 1965) and 8% (Rietschel, 1964). The latter study revealed positive patch tests in some workers without evidence of eczematous dermatitis and the prevalence of demonstrable allergy in those with symptoms was 6/152 (4%). In the United Kingdom, Keczkes & Brown (1976) found positive patch tests to 0·2% aqueous GBK in four out of nineteen workers with contact dermatitis. The factory employed 300 workers, but the number of exposed individuals was not stated.

I have investigated nine patients with hand eczema from a group of factory workers exposed to GBK in a soluble cooling oil (Castrol Clearedge EP 284). Forty-eight-hour closed patch tests were performed on the upper back in a standard way using A1-test units to which 25 μl of test solution was added. Commercially available GBK concentrate was diluted in water and tested in concentrations ranging from 0·2% to 10%. Positive eczematous reactions were observed on day 3 in eight of the nine patients. All eight gave positive reactions with 10% and 5% test solutions, but unequivocally positive reactions were found in only five using 2% and in only three using 1% test solutions respectively. Patch tests with the 10% solution were uniformly negative in eighteen control patients with eczema.

Rycroft (1978) recommended patch testing with a 1% solution of GBK but had failed to demonstrate allergic reactions in over thirty patients (presumably with eczema) who had been exposed. The present study suggests that the majority of sensitized patients would not be detected using a 1% solution and for this reason the prevalence of sensitivity is likely to have been considerably underestimated in previous studies. Cronin (1980) suggests that GBK is an irritant. However there was no irritant reaction in any of the control eczema patients who were patch tested with 10% GBK. I would suggest using concentrations of not less than 5% aqueous GBK for routine patch test screening.

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A cautionary note on UV fluorescent lamps

Following the replacement of the ultraviolet fluorescent lamps in a Theraktin Ultraviolet Equipment (Rank Stanley Cox) by the hospital engineering staff of a particular hospital, it gradually became evident to the physiotherapists, who were using the equipment, that the patients were not exhibiting

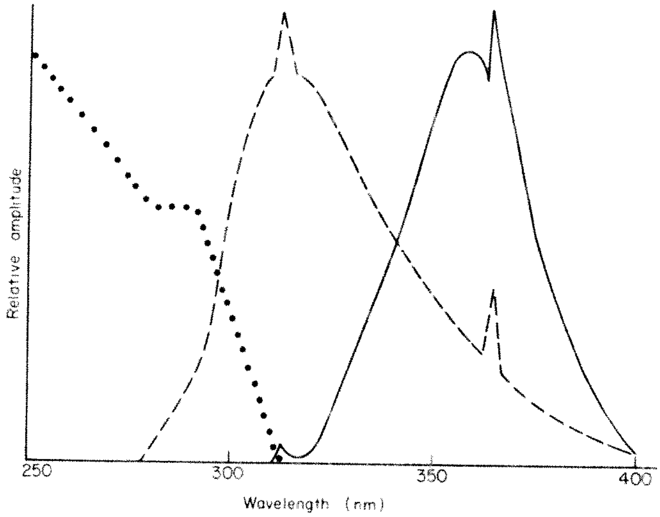

FIGURE 1. The spectral power distribution of a UV-A fluorescent lamp and UV-B fluorescent lamp, and the erythema action spectrum in human skin:——— Thorn Atlas UV lamp ('UV-A'); - - - - Philips TL12 lamp ('UV-B'); . . . . Erythema Action spectrum. (From Everett, M.A., Sayre, R.M. and Olson, R.L., 1969. Physiologic response of human skin to ultraviolet radiation. In: *The Biologic Effects of Ultraviolet Radiation with Emphasis on the Skin*, Ed. by F.Urbach. Pergamon Press, Oxford.)

the slightest degree of erythema even with unduly long exposure times. The new lamps were 'Thorn Atlas UV' and visually had the same bluish-white appearance as the old lamps. Determination of the spectrum produced by these lamps with a scanning monochromator revealed that the new lamps were emitting virtually no erythemally-effective UV radiation (UV-B and UV-C) but only long-wave UV radiation, or UV-A (see Fig. 1). When the lamps were replaced with Philips TL12 fluorescent tubes, expected results were achieved since a significant fraction of the spectrum emitted by these lamps is in the UV-region and is efficiently matched to the erythema action spectrum as shown in Fig. 1.

All fluorescent tubes designed to produce their fluorescence spectrum in the ultraviolet appear visually the same bluish-white when switched on (the exception is the so-called 'Blacklight' lamp which incorporates a filter to absorb the visible radiation). However, depending upon whether the emission spectrum is in the UV-B or UV-A will drastically alter the biological effectiveness of the lamp since UV-B is about one thousand times more effective in producing an erythema in human skin than UV-A.

Examples of fluorescent lamps which emit significant quantities of UV-B include the Philips TL12 and Westinghouse FS sunlamp. UV-A fluorescent tubes, which are used in photochemotherapy (PUVA) treatment units, include the Philips TL09, Thorn Atlas UV, Sylvania FR90T12 and General Electric F72T12/BL/HO.

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B.L.DIFFEY

British Journal of Dermatology (1981) 104, 609.

Book Reviews

Haar und Haarkrankheiten. Edited by C.E.ORFANOS (1979) Stuttgart: Fischer. Pp. xxvii + 1103. 618 figs, some in colour.

It is more than twenty years since the publication in Europe or in the United States of any comprehensive text book on the hair. During these decades advances in electron-microscopy, in microbiology in immunology and in human and comparative endocrinology in particular, have not only added immeasurably to our factual knowledge of the hair and its diseases, they have replaced many long accepted beliefs by totally new concepts soundly based on experimental evidence. Apart from its very considerable importance in clinical dermatology, the hair is now the object of extensive research by workers in many other disciplines. It is for both clinicians and research workers that Professor Orfanos of Berlin, well known for his own contributions in this field, has planned and edited this book.

The sixty-two contributors to this book are drawn from many countries and include a substantial proportion of those who have most effectively contributed in recent years to the periodical literature on the hair. Almost all the chapters provide clear and authoritative accounts of their subjects with an extensive bibliography, and can be strongly recommended. However as the Editor admits in his foreword, he has had to shorten some chapters and amplify others in order to impose some degree of uniformity on a heterogeneous collection of individual contributions. In this he has to a large extent succeeded but there is still considerable overlap and repetition. For example the chapter on diseases of the hair in childhood is excellent in itself, but contains little that is not considered more fully in other chapters. The chapter on follicular mucinosis is an admirably complete review of this disorder but it occupies nineteen pages. The chapter on the pathogenesis of hirsutism, on the other hand, occupies only thirteen pages of which three are devoted to references. Other examples could be given of an apparent lack of balance but this defect, which is after all a tribute to the Editor's kindness of heart, is readily eliminated in a second edition. We should be grateful to Professor Orfanos for providing us with an invaluable and badly needed book which will remain a landmark in the scientific study of the hair and its diseases. It is indispensable for the reference library and for the clinical dermatologist with special interest in the hair.

ARTHUR ROOK

Dermatology. G.K.STEIGLEDER and H.I.MAIBACH (1980) Chicago: Year Book Publishers Inc. Stuttgart: Georg Thieme Verlag. Pp. xii + 467. Price £12.25.

The stated aim of the authors is to cover the field of dermatology and venereology with both general physicians and beginners in dermatology in mind. In this aim the book succeeds admirably. The summary at the end of each section is both useful and easy to find as it is printed on a grey background. The black and white illustrations are of generally good quality. The clinical data presented are clear, concise and readable and the sections on management are up-to-date and largely non-controversial. The English translation is generally good and despite an occasional quaintness of idiom the meaning is always clear. The book is published in durable flexiback covers. Its price, however, may preclude its general use by medical students, but it should prove popular to physicians in training and trainee dermatologists.

J.A.COTTERILL

British Journal of Dermatology (1981) **104**, 610.

News and Notices

Fourth Investigative Techniques in Dermatology Course

The 4th Investigative Techniques in Dermatology Course will be held at the Welsh National School of Medicine, Cardiff on 30 September–2 October 1981. This course is designed to help those in training and will be of particular interest to registrars, senior registrars and others interested in investigative techniques applicable to dermatology. The emphasis at this course is on some of the basic research techniques and modern methods for the investigation of patients with skin disease and their applicability to different situations.

For further details and registration forms please contact: Professor R. Marks, Professor of Dermatology, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN.

Hawaiian Dermatology Seminar

This limited enrolment Course in Dermatology is oriented primarily to dermatologists involved in patient care and will be held in Maui, Hawaii, 5–10 February, 1982.

It is sponsored by the Northwestern University, Cleveland Clinic, and University of California (San Francisco) Departments of Dermatology. Speakers include faculty of these departments and guest faculty; a partial list of the latter includes: D.Burrows, J.Grant, K.Holubar, H.Ippen, J.Kim, A.Kopf, M.Orkin, F.Parker, D.Vander Ploeg, P.Robbins, R.B.Rees, J.Strauss and J.Tindall.

For registration information and a preliminary programme, write to: Department of Dermatology, Northwestern University Medical School, 303 E. Chicago Ave, Evanston, ILL 60201, U.S.A.

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Edited by Martin M. Black
Institute of Dermatology, St. John's Hospital for Diseases of the Skin, London, England

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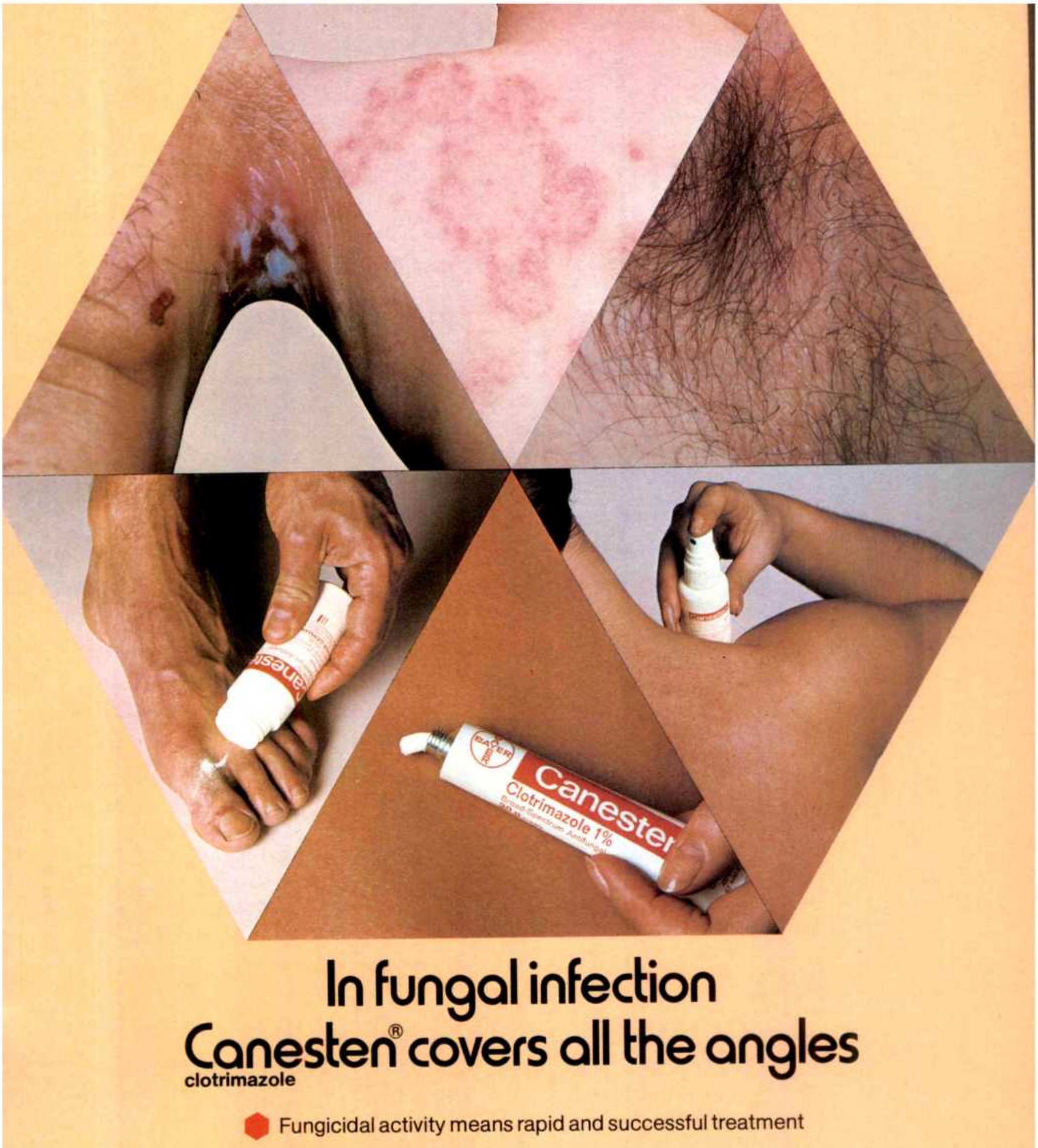

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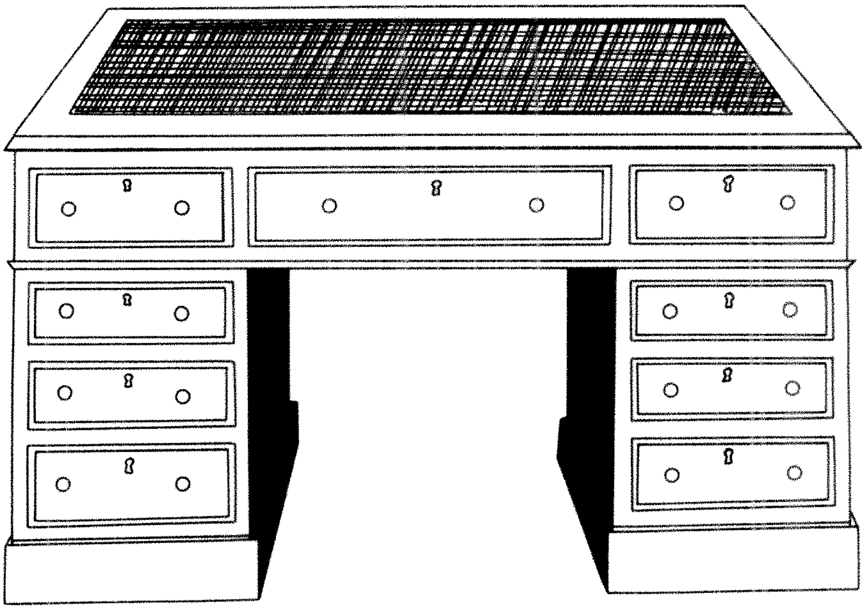

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	µg	cubic millimetre(s)	mm³	micrometre(s)	µm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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British Journal of Dermatology, Volume 104, Number 5, May 1981

CONTENTS

Clinical and Laboratory Investigations

PAGE

Discoid lupus erythematosus-like lesions and stomatitis in female carriers of X-linked chronic granulomatous disease 495
FLEMMING BRANDRUP, CHRISTIAN KOCH, MICHAEL PETRI, MORTEN SCHIØDT AND KIRSTEN STAEHR JOHANSEN

Loss of β_2 microglobulin from the cell surface of cutaneous malignant and premalignant lesions 507
MARLYN L.TURBITT AND RONA M.MACKIE

The presence of β_2 microglobulin on the membrane of the keratinocyte in premalignant skin disorders 515
H.R.KORTHALS ALTES-LEVY VAN VINNINGHE AND H.A.M.NEUMANN

Human dermal microvasculature: II. Enzyme histochemical and cytochemical study 521
J.C.HIGGINS AND R.A.J.EADY

Biochemical characterization of tyrosinase inhibitors using tyrosinase binding affinity chromatography 531
GENJI IMOKAWA AND YUTAKA MISHIMA

An action spectrum for 8-MOP induced sunburn cells in mammalian epidermis 541
A.R.YOUNG AND I.A.MAGNUS

Absorption of ultraviolet radiation by skin surface lipid 549
P.C.BEADLE AND J.L.BURTON

Herpes gestationis and anti-HLA immunization 553
E.EBERST, M.M.TONGIO, B.EBERST, S.MAYER AND A.BASSET

The association between lichen sclerosus et atrophicus and HLA-B40 561
CHRISTINE I.HARRINGTON AND K.GELSTHORPE

An investigation into the incidence of auto-immune disorders in patients with lichen sclerosus and atrophicus 563
CHRISTINE I.HARRINGTON AND I.R.DUNSMORE

Case Reports

Idiopathic pyoderma gangrenosum and impaired lymphocyte function: failure of azathioprine and corticosteroid therapy 567
S.M.BREATHNACH, G.C.WELLS AND H.VALDIMARSSON

Porphyria cutanea tarda (PCT) in a patient with chronic renal failure on haemodialysis 575
JACK R.LICHTENSTEIN, ELIZABETH J.BABB AND BERTRAM F.FELSHER

Porphyria cutanea tarda in a haemodialyzed patient 579
G.C.TOPI, GANDOLFO L.D'ALESSANDRO, G.C.CANCARINI, F.DE COSTANZA, D.GRISO AND M.RAVELLI

Cutaneous ultrastructural diagnosis of ceroid-lipofuscinosis 581
MASAMITSU ISHII, KUNIAKI TAKAHASHI, TOSHIO HAMADA, AKEMI TANAKA AND SHINOBU HIGAMI

Cerebriform tongue—a clinical sign in pemphigus vegetans 587
S.PREMALATHA, S.JAYAKUMAR, PATRICK YESUDIAN AND A.S.THAMBIAH

Cerebellar ataxia and sensory neuropathy associated with dermatitis herpetiformis 593
TAWFIQUE K.DANESHMEND AND CHRISTOPHER GARDNER-THORPE

Brief Communications

Unsuccessful treatment of alopecia areata with dapsone 597
P.S.FRIEDMANN

Juvenile plantar dermatosis in an adult 599
D.B.CZARNECKI, P.S.COWEN AND T.J.CONNORS

Comment

The current status of histamine receptors in human skin: therapeutic implications 601
M.G.DAVIES AND M.W.GREAVES

Correspondence 607

Book Reviews 609

News and Notices 610

British Journal of Dermatology

The British Journal of Dermatology is owned by and is the official organ of the British Association of Dermatologists. It is also the official organ of the Nederlandse Vereniging van Dermatologie en Venereologie (Netherlands Association for Dermatology and Venereology).

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REFERENCE:
British Medical Journal, 1978, **2**, 84.

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FOOD ALLERGY: New Perspectives *edited by* **John W. Gerrard,** *Univ. of Saskatchewan, Saskatoon, Saskatchewan, Canada. (15 Contributors)* Food allergy, its mechanisms, its effects on various organ systems, and its medical management are explained by an international consort of experts. Dermatologists will find the chapters on atopic eczema, genetic factors in food allergy of early childhood, and prevention of food allergy of special interest. *'80, 328 pp., 31 il., 26 tables, cloth-$28.50, paper-$19.75*

MORPHOLOGICAL DERMATOLOGY: A Study of the Living Gross Pathology of the Skin *by* **Robert Jackson,** *Ottawa Civic Hospital, Ottawa, Ontario, Canada. Foreword by* **Barney Usher.** Each of this text's twenty-six chapters describes a particular morphological lesion along with its various forms, method of examination, and diagnostic approach. Erythema, urticaria, purpuras, telangiectases, eczemas, bullous dermatoses, papules, nodules, ulcers, keratoses, dyschromias, cutaneous atrophies and dystrophies, onychoses, cysts, and burrows are among the many specific lesion groups explored. *'79, 368 pp. (6 3/4 x 9 3/4), 88 il., 5 tables, $34.50*

CHEMOSURGERY: Microscopically Controlled Surgery for Skin Cancer *by* **Frederic E. Mohs,** *Univ. of Wisconsin Medical School, Madison, Wisconsin.* Written by the originator and developer of chemosurgical techniques, this volume comprehensively explains methods for the microscopically controlled excision of skin cancer and provides practical information on the chemosurgical treatment of noncancerous growths. A review in ***JAMA*** stated that this book "provides the chemosurgeon a reference text for the practice of this specialty, unparalleled by other volumes." *'78, 400 pp. (6 3/4 x 9 3/4), 834 il., 59 tables, $49.75*

NAIL DISEASES In Internal Medicine *by* **Pietro de Nicola,** *Univ. of Pavia, Pavia, Italy, and* **Mario Morsiani** *and* **Giorgio Zavagli,** *both of Univ. of Ferrara, Ferrara, Italy.* The initial chapter of this book covers anatomy, histology, embryology, chemical composition, and anatomy of the nail. Remaining chapters provide clear description of specific nail disorder symptoms associated with many different categories of disease. *'74, 128 pp., 18 il. (8 in color), $14.75*

THE HOUSEHOLD ENVIRONMENT AND CHRONIC ILLNESS: Guidelines for Constructing and Maintaining a Less Polluted Residence *edited by* **Guy O. Pfeiffer,** *Link Clinic, Mattoon, Illinois, and* **Casimir M. Nikel.** *Foreword by* **Richard Mackarness.** *(10 Contributors)* The relationship between the domiciliary environment and chronic illness is analyzed and explained. Instructions for ridding the home of molecular particulates that cause various localized and systemic allergic reactions in susceptible persons are provided. *'80, 208 pp., 15 il., $14.75*

DERMABRASION AND CHEMICAL PEELING: In the Treatment of Certain Cosmetic Defects and Diseases of the Skin *by* **James W. Burks.** *Foreword by* **Morris Waisman.** This text contains all of the information necessary for performance of the operation plus a firm scientific foundation based on original serial biopsies and other studies by the author. Dermal abrasion, wire brush planing, comparison of dermabrasion and chemical peel, and anesthesia by refrigeration are only a few of the many topics covered. *'79, 256 pp., 192 il., 5 tables, $24.75*

INTRODUCTION TO MODERN PHOTOTHERAPY *by* **Leon Goldman,** *Univ. of Cincinnati, Cincinnati, Ohio.* This book demonstrates how different portions of the electromagnetic spectrum can provide therapeutic modalities. Discussions are presented on ultraviolet and infrared light, phototesting, instrumentation, and safety. Phototherapeutic applications for psoriasis, acne, herpes simplex, miscellaneous skin diseases, and systemic disorders are detailed. Current laser light phototherapy, light art as therapy, and transillumination also receive careful consideration. *'78, 144 pp., 42 il., 4 tables, $19.25*

HAIR TRANSPLANT SURGERY *by* **O'Tar T. Norwood,** *Univ. of Oklahoma Medical School, Oklahoma City, Oklahoma.* Following a classification of male pattern baldness, sections are included on patient selection, surgical technique, and prevention and correction of postoperative problems. Additional subjects covered include the current status of hair bearing homografts, placing the hairline, and estimating future hair loss. *'73, 148 pp. (6 3/4 x 9 3/4), 133 il. (64 in color), 7 tables, $14.75*

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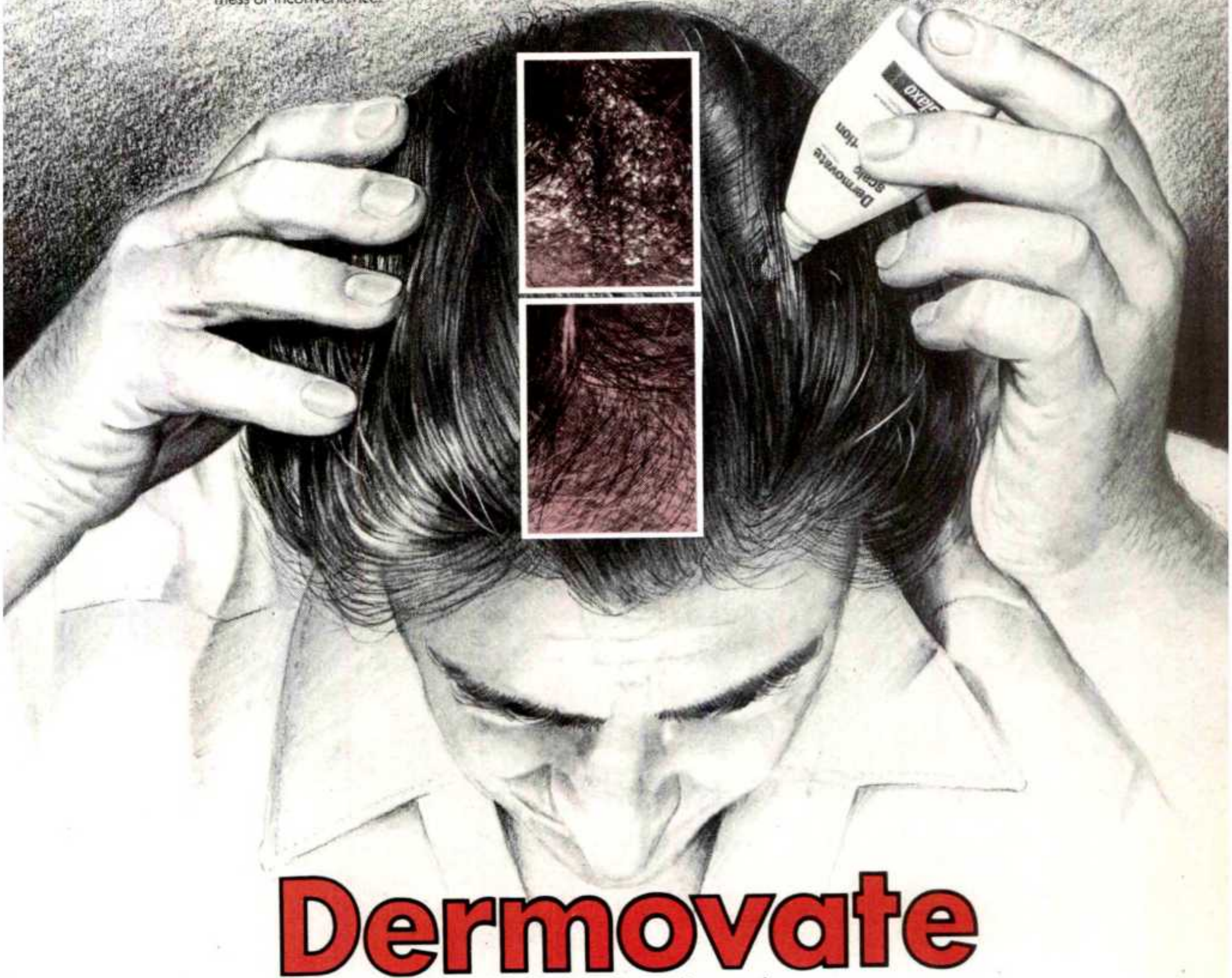

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Development of secondary infection requires withdrawal of topical corticosteroid therapy and institution of appropriate systemic antimicrobial therapy.

Topical administration of corticosteroids to pregnant animals can cause abnormalities of fetal development. In general, topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

Dermovate preparations are usually well tolerated, but if signs of hypersensitivity appear, application should be stopped immediately. As with other topical corticosteroids, local atrophy may result from prolonged treatment, particularly if occlusive dressings are used. When extensive areas are treated, sufficient systemic absorption may occur to produce the features of hypercorticism, especially in infants and children. This effect is more likely to occur after prolonged treatment, particularly if occlusive dressings are used. In rare instances, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have

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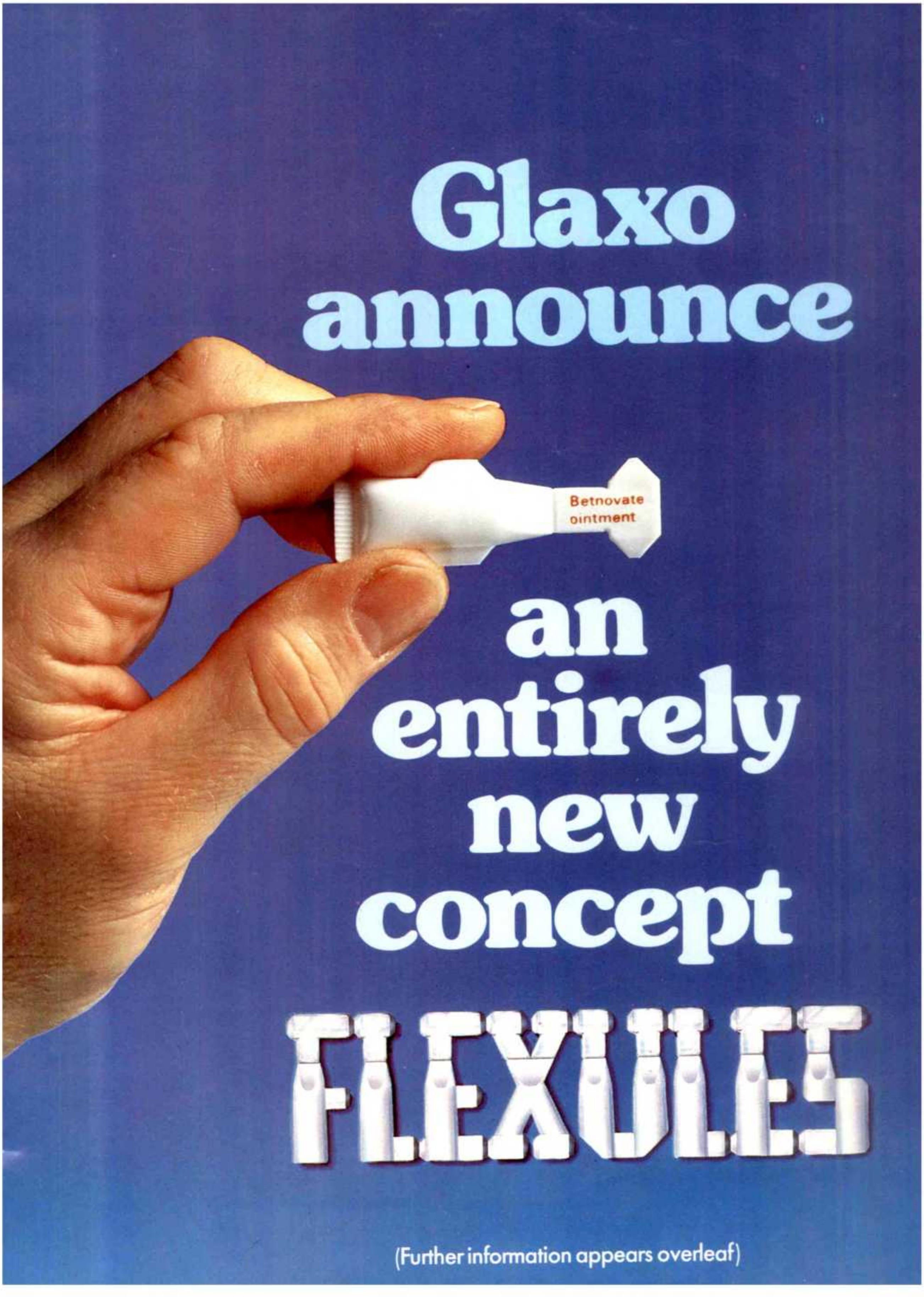

(Further information appears overleaf)

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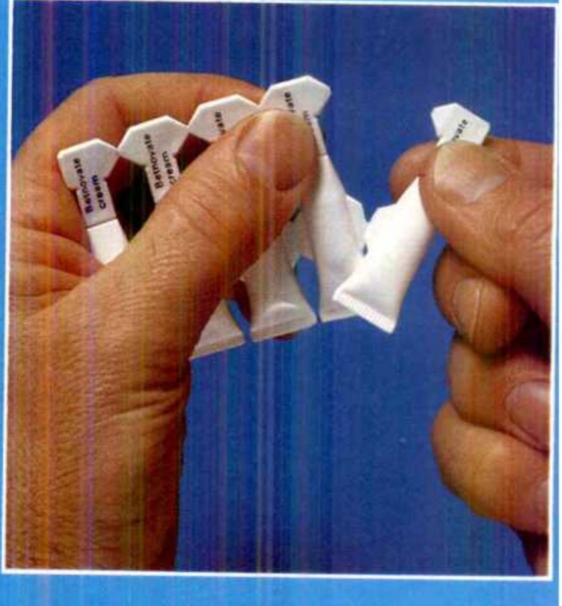

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Prescribing information

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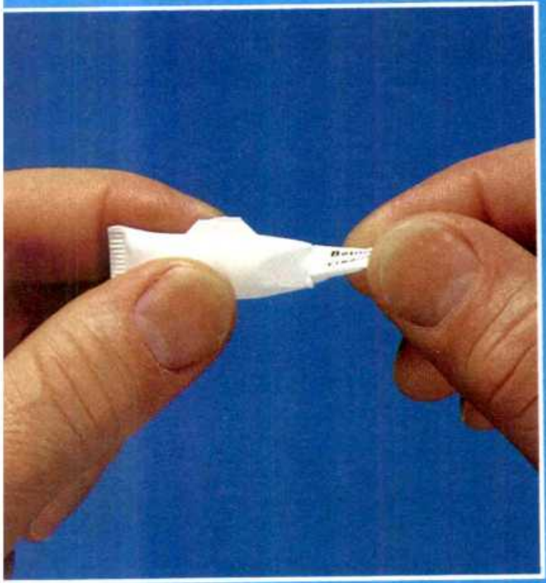
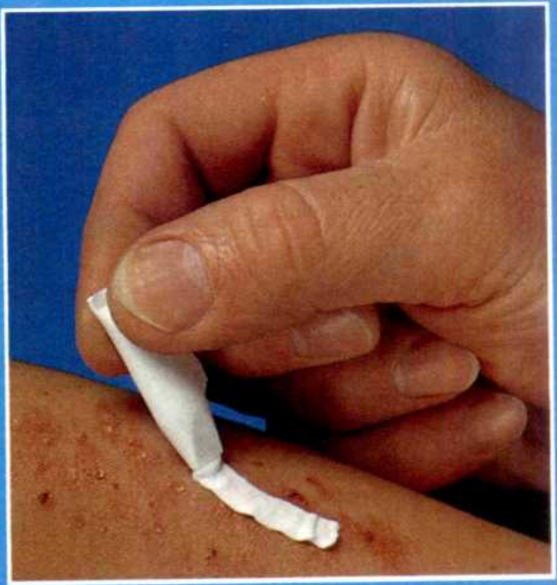
Precautions
Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit

A new measure of control in topical steroid therapy





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Contra-indications

Bacterial, fungal or viral diseases of the skin. Dermovate and Betnovate are also contra-indicated for rosacea, acne and peri-oral dermatitis. Hypersensitivity to the preparation.

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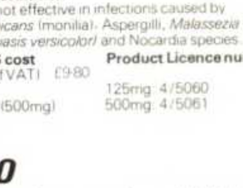

THE INSIDE STORY ON FUNGAL INFECTION

Prescribing information

Dosage and administration

Doses should be taken after meals for maximum absorption.

Adults: normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily.
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Contra-indications

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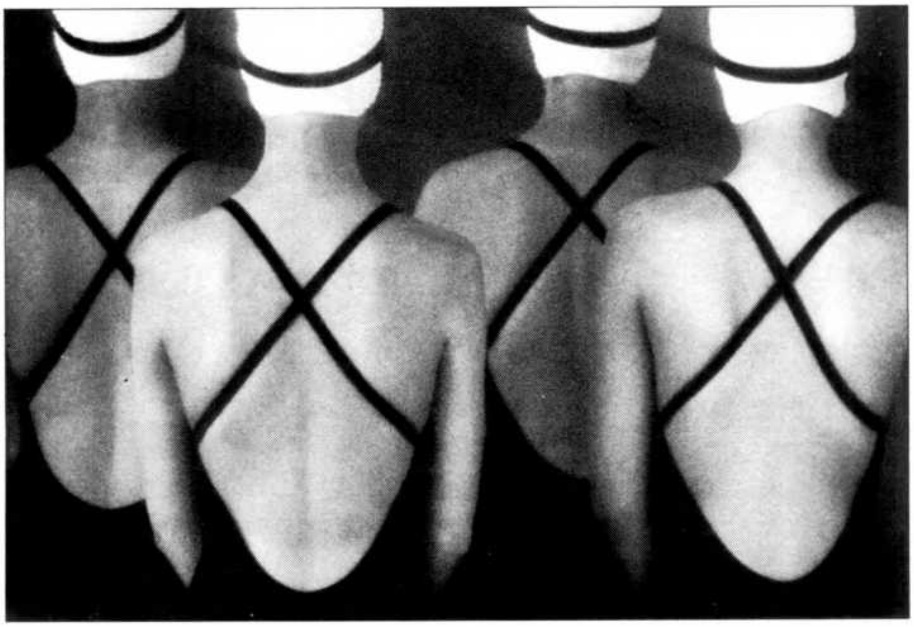

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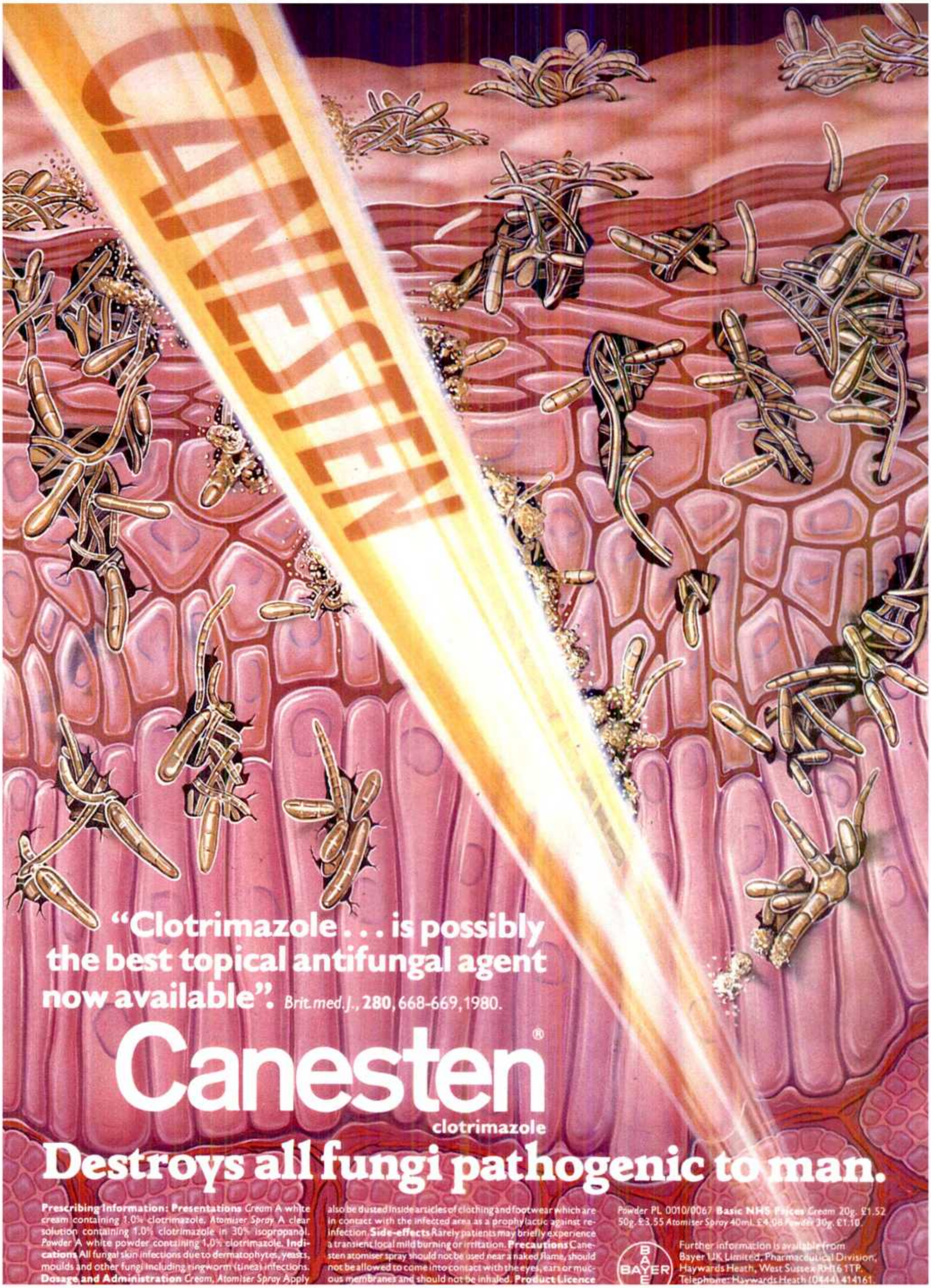
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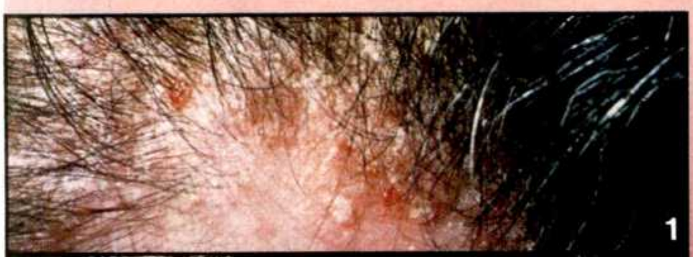

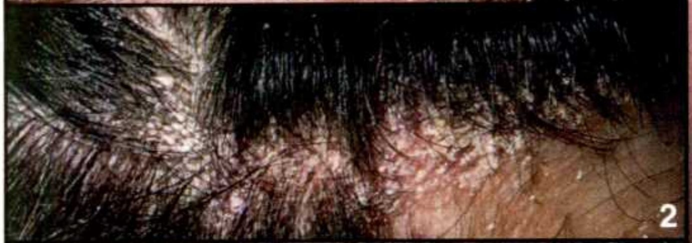

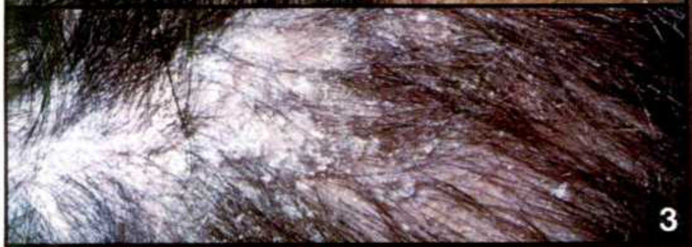

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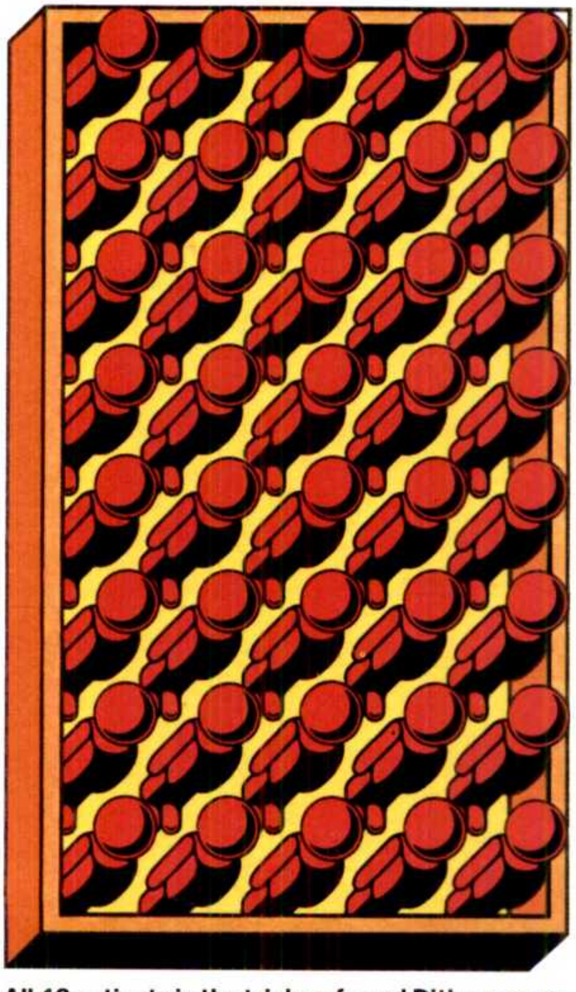

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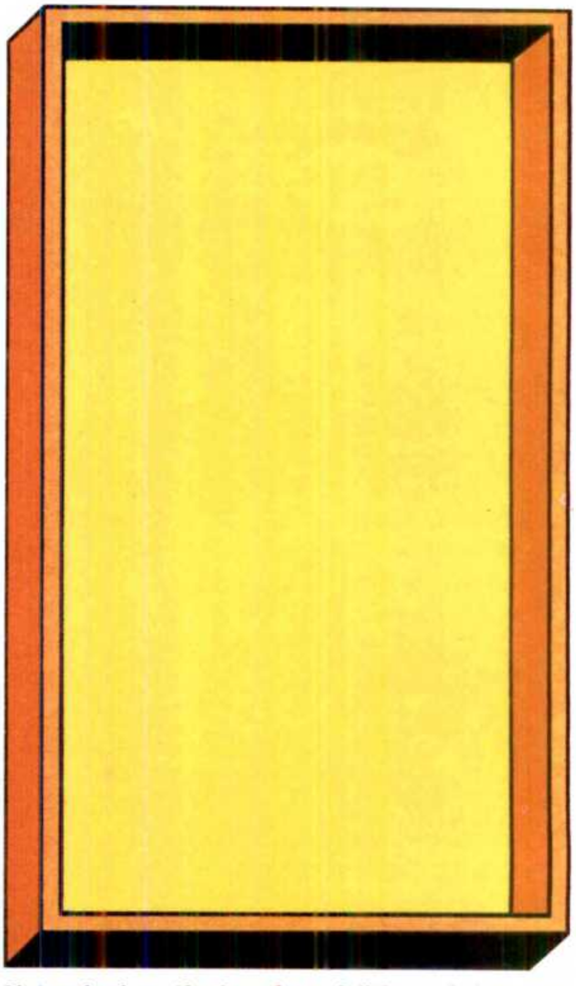

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1. *British Journal of Dermatology*, 1979, **100**, 475

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British Journal of Dermatology (1981) **104**, 611.

Clinical and Laboratory Investigations

Dermatological non-disease: a common and potentially fatal disturbance of cutaneous body image

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SUMMARY

A group of patients presenting with dermatological complaints but with no significant objective dermatological pathology on examination are described. Twenty-eight patients, twelve male and sixteen female, age range 16–76 years, mean 46 years, were seen. Symptomatology was confined to three main body areas: the face—eight patients; scalp—nine patients; perineum—eight patients. The complaints related to the face were burning, intense itching and hirsutes. Scalp symptoms included excessive hair loss and intense irritation. Genital symptoms included itching, excessive redness, burning and discomfort, which in three instances prevented the patient sitting.

A disturbed body image (dysmorphophobia) was common and the most frequent psychological illness present was depression. Two patients were demented and in two a diagnosis of schizophrenia was made. One patient committed suicide and two patients attempted suicide. Female patients presenting with facial symptoms have a more ominous prognosis, both with regard to the risk of suicide and the development of psychosis.

It is important that dermatologists recognize this common group of patients with dermatological non-disease. Those patients who are anxiously preoccupied with their skin may be managed by superficial psychotherapy and antidepressants. Those patients who are truly deluded should be referred to a psychiatrist.

The concept of non-disease was introduced by Meador (1965), who proposed that the absence of diagnosable symptoms and signs, i.e. non-disease, does not necessarily indicate an absence of significant symptomatology. A group of patients presenting with dermatological complaints but with no significant objective dermatological pathology on examination are described.

PATIENTS AND METHODS

Twenty-eight patients with dermatological non-disease, twelve males and sixteen females, age range

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16–76 years, mean 46 years, seen during the last year are described. Twenty-three of these patients were seen in private practice. Symptomatology was confined to three main body areas, the face, the scalp and the perineum. Complaints relating to the face included intense itching, burning, redness and hirsutes. Scalp symptomatology included excessive hair loss and intense irritation. Perineal symptoms were itching, excessive redness and burning, and discomfort which in three instances prevented the patient sitting. Patients with generalized pruritus and classical pruritus ani and vulvae were excluded from this study. The clinical details are given in Tables 1–4.

Patients presenting with facial symptoms

The clinical details of this group of patients are shown in Table 1. There were nine patients, six females and three males, age range 16–73 years, mean 45 years.

Patient 1. A teacher, aged 34 years, with two children, referred with a two-week history of itching and burning confined to the face and forehead. She admitted that she had been sleeping badly recently and that there had been a recent bereavement on the husband's side of the family. On examination there were no objective dermatological findings and patch tests to a variety of cosmetics were performed. The patient failed to return to have her patch tests read because she had thrown herself under a train and successfully committed suicide. Her general practitioner told me later that he thought there may have been some marital problems.

Patient 2. A female, aged 39 years, gave a history of excessive concern over facial hair following a chance remark about facial hair by her sister some months previously. Since then the patient had spent most of each day examining her face with a magnifying glass and mirrors. This compulsion frightened her and she was unable to go out. When I saw her she was seriously contemplating suicide. On examination she was tearful, but there was no significant dermatological abnormality. A final diagnosis of dysmorphophobia in an anxious perfectionist who was depressed was made by the psychiatrist who saw her.

Patient 3. A female, aged 37 years, a chemistry teacher, concerned by what she imagined to be excessive facial hair for 14 years. Electrolysis had been tried but had not helped and the patient felt her problem was ruining her life. The patient came originally from Wales and was socially isolated in North Yorkshire. She took an overdose shortly after seeing a psychiatrist, who described her as a 'bloody hysteric'. She subsequently left her husband and family for Wales. On examination there was no significant dermatological abnormality but she appeared to be depressed and a final diagnosis of dysmorphophobia and reactive depression was made.

Patient 4. A 48-year-old nurse, who presented because of her worry over excessive facial hair for 6 years. Before the patient was able to go out she had to pull out any hairs she found around her mouth and this ritual usually resulted in folliculitis. On average it would take her an hour to deal with her imagined facial hair before she could leave her house. She was very worried about what other people might say about her facial hair and was quite unable to go on holiday. She was completely isolated socially. There was a past medical history of depression which was treated with ECT in 1976. On examination, there was no significant hirsutes, but there were many small scars around her mouth which had followed the use of forceps. A final diagnosis of dysmorphophobia and depression was made.

Patient 5. A female, aged 16 years, presented with a complaint that her nose had become disfigured

TABLE I. Patients with facial symptoms

Patient	Sex	Age	Occupation	Presenting symptoms	Past history	Final diagnosis	Outcome
1	F	34	Teacher/ mother	Facial itching	Nil	Reactive depression	Committed suicide
2	F	40	Canteen worker/ mother	Excess facial hair	Nil	Dysmorphophobia, depression	Serious risk of suicide
3	F	37	Teacher/ mother	Excess facial hair	Nil	Dysmorphophobia, depression	Overdose, left husband and family
4	F	48	Housewife ex-nurse	Excess facial hair	ECT for depression 1977	Dysmorphophobia, depression	Psychotherapy, still very limited by symptoms
5	F	16	Unemployed	Spots on nose	Recent overdose	Dysmorphophobia, schizophrenia	Serious risk of suicide, psychiatric care
6	F	66	Housewife	Burning face	Depression	Endogenous depression, dysmorphophobia	Still depressed, poor response to antidepressants, no response to pimozide
7	M	73	Retired decorator (married to patient 6)	Burning face	Depression	Endogenous depression, dysmorphophobia	Poor response to antidepressants, no response to pimozide
8	M	21	Teacher	Facial rash, later hair loss	Nil	Dysmorphophobia, anxious perfectionist	Health addict, symptoms followed genital herpes infection, back injury
9	M	70	Retired	Lumps on ears	Depression, cancer phobia, sore scrotum	Dysmorphophobia, depression	Antidepressants

after she had steamed her face over a bowl of hot water. Since this event the patient had been constantly preoccupied with her nose, and the nose of everyone else she saw, including people on television. This preoccupation stopped her going out and working. She had recently taken an overdose because of her worries about her nose and felt plastic surgery would solve all her problems. There was a family history of schizophrenia. There was no response to a variety of topical medication. On examination there was no significant dermatological abnormality and a final diagnosis of dysmorphophobia due to schizophrenia was made. The attending psychiatrist felt that there was a definite risk of suicide in this patient. My fee was not paid.

Patients 6 *and* 7. Husband and wife, aged 73 and 66 years respectively, gave a 7-week history of burning face and ears. The husband was affected first, followed shortly afterwards by his wife and

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some weeks later by a son who visited the house. The patient and his wife had to sit at home during the winter with the windows and doors open and the grandchildren were forbidden to come to the house in case they 'caught it'. There was a past medical history of depression in both patients and there was no significant dermatological abnormality on examination. There was no response to topical medication, antidepressants, Dixarit or pimozide. Another opinion was sought and a diagnosis of endogenous depression was made in the husband, who responded poorly once more to antidepressants. This family took badly to my fee.

Patients presenting with scalp symptomatology

The clinical details are shown in Table 2.

There are nine patients in this group, six females and three males, age range 23–48 years, mean 35·7 years.

TABLE 2. Patients with scalp symptoms

Patient	Sex	Age	Occupation	Presenting symptoms	Past history	Final diagnosis	Outcome
10	F	38	School secretary	Hair loss	Recent hysterectomy	Depression, Dysmorphophobia	Psychotherapy, less depressed on amitriptyline
11	F	46	Company director	Hair loss	Nil	Dysmorphophobia	Later glossodynia and panic attacks
12	F	43	Housewife	Excess hair loss 5 years	Began 6 months after hysterectomy	Dysmorphophobia	Psychotherapy
13	F	40	Nurse/housewife	Excess hair loss	Nil	Dysmorphophobia, stress	Psychotherapy
14	F	27	Nanny	Excess hair loss	Nil	Dysmorphophobia	Responded well to Atarax
15	F	48	Housewife	Scalp itching	Depression, divorced 14 years	Depression	Poor response to local steroids
16	M	30	Engineer	Burning scalp	Nil	Reactive depression	Recent divorce
17	M	26	Accountant	Excess hair loss	Nil	Dysmorphophobia	Psychotherapy
18	M	23	Student	Excess hair loss	'Schizoid'	Dysmorphophobia	Doing badly at studies, psychiatric opinion

Patient 10. A secretary and housewife, aged 38 years, who complained that her hair had been falling out in handfuls for 2 years. This problem began about 3 months after a hysterectomy. Her husband was often away from home and there had been recent stress at work because of cancer in one of her employers. On examination there were no significant dermatological abnormalities. The patient was depressed and tearful, and is sleeping better on Lentizol, but is still adamant that her hair is coming out in handfuls.

Patient 11. A female company director, aged 46 years, who presented with a 10-month history of 'hair coming out in handfuls'. On examination there was no significant abnormality. The patient subsequently presented complaining of a sore mouth and tongue which got progressively worse as the day went on. She had recently experienced some panic attacks whilst on holiday on her own in Spain. On examination there was no significant abnormality of either scalp or mouth. A final diagnosis of dysmorphophobia, social stress and glossodynia was made.

Patient 12. A male accountant, aged 26 years, seen because of his concern over hair loss for 2½ years. His anxiety about this problem was spoiling his life and making it difficult for him to form any meaningful relationships with the opposite sex. Because of his worries he had had a prophylactic hair transplant on his forehead during a visit to London in 1979. This patient is now asking if these transplants could be ablated but the Tao Clinic has refused to do this.

He has a past medical history of 'rosacea', irritable colon and pruritus ani.

A final diagnosis of dysmorphophobia in an obsessional and anxious personality was made.

Patients presenting with genital symptomatology

The clinical details are shown in Table 3.

TABLE 3. Patients with genital symptoms

Patient	Sex	Age	Occupation	Presenting symptoms	Past history	Final diagnosis	Outcome
19	M	68	Retired	Perianal discomfort	Nil	Dementia	No response to local measures
20	F	76	Housewife	Severe discomfort in perineum	Anxiety, depression, burning	Depression, ? dementia	Multiple consultants, no response to any medication including Pimozide
21	M	41	Sales manager	Pain at end of glans penis	VD phobia	Dysmorphophobia, reactive depression	Multiple consultants
22	M	38	Shop manager	Redness of scrotum, genital and crural discomfort	Nil	Dysmorphophobia	Followed candida infection
23	M	65	Retired	Red, painful scrotum	NSU 1977	Dysmorphophobia	Improved with tranquillisers
24	M	60	Civil servants	Red, sore scrotum (is it a germ ?)	Nil	Dysmorphophobia	Improved on Lentizol
25	F	60	Tailoress	Itchy perineum and breasts	Anxiety	Stress	Some response to Atarax and local measures
26	M	54	Artist	Pain in perineum	'Prostatitis' exploration groin for crural discomfort	Social stress	Multiple consultants

There are eight patients in this group, two females and six males, age range 38–76 years, mean 57·8 years.

Patient 13. A retired designer, aged 68 years, accompanied by his wife, who gave most of the history. The patient had been seen recently by two consultant surgeons because of discomfort in his perineum which had been treated by anal dilatation, but his symptoms became worse after this. Some small internal piles had recently been injected causing an exacerbation of his symptoms. He was preoccupied with his bowels which he said were opened six times a day. On examination, he was attired in a bright purple vest which extended to his knees and he declined to sit down because of his discomfort. He was plainly demented and a final diagnosis of arteriosclerotic dementia was made.

Patient 14. A female, aged 76 years, with a history of a red, sore face in 1974, for which she received tetracycline with a presumptive diagnosis of rosacea. Subsequently her mouth and tongue became sore and she was treated for a candida infection, but the soreness persisted, despite a wide variety of local anti-candida agents. All investigations, including serum vitamin B12, folates and serum iron were all normal. Antidepressants were eventually tried, but these made her mouth more dry and sore. In August 1978 the patient had a small stroke and fell, with trauma to her coccyx. Since that event she has experienced a burning pain in the perineum, upper legs, lower abdomen and buttocks which prevents her sitting down or sleeping. She carries a padded air ring with her wherever she goes and is preoccupied with her bowels. She has seen a multiplicity of specialists, including several dental surgeons, two neurologists, two gynaecologists, one general surgeon, one urologist, one pain-relief anaesthetist and one psychiatrist. She has shown no response to any medication at all, indeed, most medication makes her worse. The patient is very loyal, and is usually accompanied by her husband, who makes copious notes of her problems. There has never been any significant dermatological abnormality. The final diagnosis is in doubt and lies between a depressive illness and early dementia.

Patient 15. A civil servant, aged 60 years, complained of redness and burning of the scrotum which developed shortly after a holiday in Majorca, which, he said was surrounded by 'dirty sea'. The patient was worried that he had been infected following bathing in the Mediterranean. His general practitioner had treated him for a minor dermatitis of the scrotum with local steroids but these measures had made him worse. Bland local emollients such as Natuderm also made him worse. Whilst taking amitriptyline he said, 'I am almost accepting it as normal now'. On examination, there was no significant abnormality at any time, apart from a small varicocele. The patient was very obsessional and washed his hands after he had demonstrated his genital area to me. His constant worry was that he may have acquired an infection and this worry was still with him when he asked me to send my account to his office rather than to his home so that his wife didn't find out about his problems.

Patient 16. An entrepreneur and artist, aged 54 years, referred for a dermatological opinion because of perianal warts and also because of severe pain in the perineum and rectum for which no cause could be found. His symptomatology began in the early 1960's when a presumptive diagnosis of chronic prostatitis had been made but never substantiated. His perineal symptoms failed to respond to a wide variety of drugs, including sedatives and antidepressants. Finally he persuaded an orthopaedic surgeon to explore both groins to try and relieve the discomfort in his upper thighs but all to no avail. Subsequently he developed back pain, pain around one knee-joint and pruritus ani. Since 1968 he has consulted four different orthopaedic surgeons, one dermatologist, one oral surgeon, one urologist, one rheumatologist, one neurologist, one physician with an interest in neurology, one general physician, two ENT surgeons, at least one venereologist, and one ophthalmic surgeon. The only significant pathology that has ever been found in the last 12 years has been a few genital warts and a small papilloma of bladder. The patient has remained particularly loyal, especially to his urologist and also to one of the physicians.

TABLE 4. Miscellaneous patients

Patient	Sex	Age	Occupation	Presenting symptoms	Past history	Final diagnosis	Outcome
27	F	57	Housewife	Itense itching on arms	Neurotic	Anxiety state Marital stress	Little response to local or oral therapy
28	F	51	Secretary	Excessive sweating in in axillae	Rhinoplasty for cosmetic reasons Hysterectomy 1 year ago	Dysmorphophobia	No response to local measures

DISCUSSION

It is important to realise that disease can exist without producing illness and illness can occur without evident disease. Dermatologists are trained to recognize disease largely by a process of pattern recognition and thus the concept that there can be significant disease in the absence of demonstrable skin pathology is easy for the dermatologist to grasp, for instance in regard to generalized pruritus and its investigation and pathogenesis.

The symptomatology in these patients with non-disease was confined, with two exceptions, to the scalp, face and perineum. The most common symptoms referable to the face were complaints of itching and burning and a preoccupation with imagined facial hair was common. This group of patients was predominantly female and seemed to have the most ominous prognosis. One female with a burning and itching face committed suicide; one female preoccupied with imagined excessive facial hair attempted suicide, as did one girl with a delusion about an imagined damaged nose. Another patient with imagined facial hair was frankly suicidal.

Scalp symptomatology included intense itching, often coupled with a complaint that the hair was coming out in handfuls and usually accompanied with a morbid fear of impending baldness. There was again a female preponderance in this group of patients. Suicide did not feature in this group but depressive illness was the commonest final psychiatric diagnosis.

Perineal symptomatology included severe discomfort, making it impossible to sit down and this symptom was present in three patients. Male patients were much more common in this generally older group and symptoms often developed after frank or imagined exposure to potential infection and concern about venereal disease. The main complaints in males were of an uncomfortable, red and inflamed scrotum, with symptoms often extending to the upper anterior thighs. One characteristic feature in these patients was the failure to respond to a wide range of topical and oral therapy and frequent precipitation of symptoms following trauma to the area. These patients also tended to collect consultants in a wide range of specialties but suicide was not a feature.

It is interesting that two of the patients also had glossodynia at some stage of their illness. This symptom may be a feature of depression, when it is often very difficult to eradicate. It is also interesting that the patient who complained of excessive sweating in her axillae had also had a recent rhinoplasty for cosmetic reasons one year previously. The high incidence of subsequent schizophrenia in patients who have undergone rhinoplasty for aesthetic reasons has been stressed by Connolly & Gipson (1978).

The majority of patients described in this paper were seen in private practice and this may reflect their anxiety about being rejected in an NHS situation. Although the syndrome seems to be common, there are relatively few relevant data in the dermatological literature. However, Eckert (1975) suggested that women who complain of hair loss which seemed to be disproportionate to the objective degree of alopecia present should be questioned for symptoms of depression and marital difficulties. Moreover, Sneddon (1979), in an account of psychiatric illness presenting to the dermatologist, described some patients with dermatological symptomatology, but little or no objective dermatological pathology. Sneddon also stressed that dermatologists should be capable of recognizing psychoneurosis and psychosis when confronted with it.

The syndrome of dermatological non-disease should be diagnosed on positive grounds, and the patient should have no demonstrable dermatological disease but will usually have symptomatology confined to the face and tongue, scalp, perineum and, more rarely, axillae. The most common psychiatric disturbance in the present series was depression, but there was often a poor response of the dermatological symptomatology to treatment with antidepressants. Many of the patients could be given an initial diagnostic label of 'dysmorphophobia' (Morselli, 1886; Connolly & Gipson, 1978; Hay, 1970). This term may be used to describe a complaint of some physical defect which the patient thinks is noticeable to others, but in reality there are no objective changes on examination. Dysmorphophobia is really concerned about a disturbance in psychological body image, and in dermatological non-diease there may be a fundamental disturbance in cutaneous body image. In this regard the face and the nose in particular seem to be central in an individual's concept of his body image (McDonald Critchley, 1979). It is not surprising therefore that imagined pathology of the face, scalp and nose in particular may be associated with profound psychiatric pathology.

A distinction between monosymptomatic hypochondriacal psychosis, a psychotic illness, and dysmorphophobia, which some regard as a neurotic type of disturbance (Munro, 1980), may not always be easy to make. Whilst patients with monosymptomatic hypochondriacal psychosis seem to respond well to pimozide, patients with nonpsychotic hypochondriacal disorders do not (Munro, 1980). The writer, however, has had no success with pimozide in four severely affected patients, but this drug does seem to be effective in patients with delusions of skin infestation.

Thus, management is not easy in this group of patients and is always much more time consuming than in patients with skin disease. It is usually not appropriate to respond with an immediate referral to a psychiatrist, for the patient who consults a dermatologist has already decided whose treatment he desires and will accept (Frithz, 1979). Those patients who are merely anxiously preoccupied with their skin may be managed by superficial psychotherapy and antidepressants. However, those patients who are truly deluded should be referred to a psychiatrist. Particular care should be taken in dealing with female patients with facial symptoms.

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British Journal of Dermatology (1981) **104**, 621.

Autoantibodies to an epidermal protease inhibitor in skin diseases

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SUMMARY

Solid phase radioimmunoassay for measurement of autoantibodies in human sera to an epidermal protein is presented. The protein purified from normal human epidermis is inhibitory to SH-dependent proteases and its molecular weight is about 13,000. Antibodies were detected in 139 of the 4,400 sera collected from dermatological patients. Most of the patients suffered from wide-spread eczematous conditions. It is suggested that the antibodies are secondary to the inflammatory conditions, but they may exacerbate or delay the course of the disease.

Järvinen & Hopsu-Havu (1975) and Järvinen (1976) described two inhibitors of sulphydryl dependent proteases in rat skin. The inhibitor with lower molecular weight, i.e. about 13,000, was demonstrated to be located in the epidermis and that with the higher molecular weight, i.e. about 74,000, in the dermis (Järvinen, 1978). A similar low molecular weight inhibitor in human skin (HEI) (Fräki 1976, Järvinen, 1978) is detectable in the *keratinising* cells of human epidermis and of the other epithelia but not in other tissues (Räsänen, Järvinen & Rinne, 1978). The inhibitor both in rat and human epidermis is very soluble and antigenic and, therefore, it is possible that it could stimulate formation of autoantibodies in disease conditions involving keratinizing epithelia. In the present work we report a solid phase radioimmunoassay for detection of such autoantibodies and results obtained in patients with various skin diseases.

MATERIALS AND METHODS

Preparation of epidermal protease inhibitor

HEI was purified as described by Järvinen (1978). The protein concentration of the HEI antigen solution was 16–21 μg/ml by the Lowry method (Lowry *et al.*, 1951). The preparation was stored at −20°C until used. Analytical isoelectrofocusing, preparative isoelectrofocusing and PAGE of the purified HEI preparation revealed two protein bands which both possessed inhibitory activity. Antiserum produced against HEI in rabbits formed after immunoelectrophoresis only one precipitate band.

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Production of hyperimmune serum

Antibodies were raised in rabbits by injecting 50 μg of HEI in Freund's complete adjuvant, and the specificity of the antibodies determined (Järvinen, 1978).

For rabbit antibody assays phosphate buffered saline, pH 7·35 (PBS) supplemented with 20% normal sheep serum and 2% Tween 20 was used as the serum diluent.

Serum samples

The human serum specimens examined comprised 4,400 samples obtained from the patients admitted to the Department of Dermatology, University Hospital of Turku, during the years 1974–1978 for different skin diseases. The sera were not selected according to dermatological diagnosis, age or sex of the patients. The sera were stored at −20°C until used in the assays. For the HEI antibody assays sera were diluted to 1:50 or 1:64 with PBS supplemented with 1% bovine serum albumin fraction V and 0·5% Tween 20. Serum samples obtained from 100 healthy adult persons served as controls.

Labelled antibodies

The heavy chain specific antibodies from the antihuman IgA, IgM and IgG immunoglobulins (Orion Diagnostica, Helsinki, Finland) were isolated by immunoabsorbent column chromatography. Antibodies were iodinated by the method of Hunter & Greenwood (1962). Specific activities of the labelled immunoglobulins ranged from 5 to 20 μCi/μg. The amount of iodinated antibodies used in the assays was standardized to give 2,500 c.p.m. when 0·2 ml of the labelled antiserum was incubated for 1 h at 37°C with a polystyrene ball which had adsorbed 2 μg of purified human IgA, IgM or IgG immunoglobulins. In the assays Eagle's minimum essential medium supplemented with 0·5% lactalbumin hydrolysate and 10% heat inactivated calf serum was used as the diluent.

The antirabbit IgG was formed by immunizing sheep and purifying the specific anti-immunoglobulins by column chromatography. It was labelled and standardized as above.

Radioimmunoassay for HEI antibodies

The solid phase radioimmunoassay (RIA) used in this study was originally developed to detect antiviral IgG or IgM antibodies (Kalimo *et al.*, 1976). In this study the same basic RIA method was used with the exception that the polystyrene balls (diameter 6 mm, the solid phase for the RIA) were coated with HEI instead of viral antigens. For coating the balls were incubated submerged in 1:100 HEI solution overnight at room temperature. The balls were then air dried and stored at 4°C until used.

For the assays a 200 μl aliquot of the serum dilutions was pipetted on the antigen coated polystyrene ball in a 4 ml disposable plastic tube. After incubation for 1 h at 37°C the serum dilution was aspirated off and the ball was washed with tap water. Then 200 μl of the I^{125} labelled antihuman IgG (A or M) was added to the tubes and the balls were additionally incubated at 37°C for 1 h (overnight for IgM). The radioactive solution was then removed and the balls were washed with tap water, removed to clean plastic tubes and the radioactivity bound was counted with a LKB Wallac 1280 gamma counter.

A positive and negative control serum, the hyperimmune and normal rabbit serum and appropriate buffer blanks were included in each test. The specimens in which the c.p.m. value was repeatedly more than 100 were regarded as positive.

RESULTS

Radioimmunoassay for epidermal protease inhibitor antibodies

The optimal test conditions were established with the rabbit serum samples used as positive and negative control reagents.

The optimal coating of the polystyrene balls with HEI was found to be on antigen dilution of 1 : 100. With lower dilutions the difference in c.p.m. values between positive and negative samples was practically the same, generally 30-fold, but there was much loss of antigen. Higher HEI dilutions resulted in obvious decreases in the c.p.m. values.

The incubation solutions and incubation times for rabbit antibody assays were based on earlier viral antibody assays. However, on repeated tests the reproducibility was found good and the difference in c.p.m. values between positive and negative specimens remained in the same range.

Storage of the antigen coated polystyrene balls decreased the specific activity, and thus ready made balls had to be used within a week after coating. Freezing and thawing of the HEI solution before coating was also deleterious. Therefore, the antigen should be divided into small portions to be used immediately for coating.

Dilution of the serum specimens or the labelled immunoglobulins with PBS supplemented with 20% normal sheep or pig serum and 2% Tween 20 did not improve the results.

The c.p.m. values for the human serum specimens were low as compared with the hyperimmune rabbit serum. However, it was evident that the specimens could be divided into two groups with the cut off line of 100 c.p.m. All specimens with counts of 100 c.p.m. or more were retested before they were accepted as positive. In addition, seventy specimens were tested as duplicates. There was good agreement in results and the remainder of the specimens were tested as single determinations. The mean c.p.m. value for 100 specimens from healthy controls was 26 c.p.m., with an SD 23 c.p.m. The total range for the positive specimens was 100–558 c.p.m. which was low as compared with the 2,500–3,000 c.p.m. for the immunized rabbit specimen at the same dilution. The total distribution of the c.p.m. values of 139 IgG positive and of 100 healthy control patients are illustrated in Fig. 1, in which the means and standard deviations are also indicated.

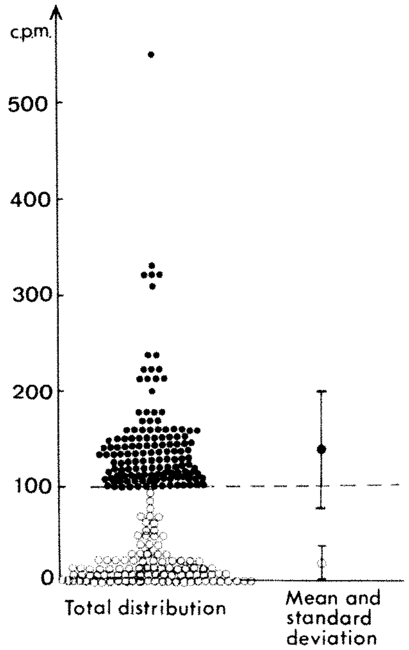

FIGURE 1. Distribution of c.p.m. values and means and standard deviations for 139 positive (closed marks) and 100 control (open marks) samples

Clinical study

Anti HEI IgG RIA antibodies were detected in 139 (3·2%) out of 4,400 specimens tested. No correlation could be found between the occurrence of these antibodies and the age or sex of the patients. In general, the patients with HEI IgG antibodies suffered from wide-spread dermatitis, psoriasis or autoimmune disease with extensive skin manifestations.

Patients with extensive dermatitis represented 68% of the positive material (Table 1). No IgA or Igm HEI RIA antibodies could be detected.

TABLE 1. The clinical diagnosis of the 139 patients with IgG RIA antibodies to human epidermal protease inhibitor among the tested 4,400 sera

Diagnosis	Number of patients		Per cent	
Dermatitis		95		68
Associated bacterial infection	29		31	
Associated candidosis	9		9	
Associated viral infection	3		3	
Atopic dermatitis	9		9	
Others	45		47	
Urticaria chronica		8		6
Psoriasis vulgaris		18		13
Erythema multiforme		11		8
Autoimmune diseases (vasculitis, collagenosis, pemphigus foliaceus and vulgaris, pemphigoid, subcorneal pustular dermatosis, dermatitis herpetiformis)		7		5
Total		139		100

DISCUSSION

The human epidermal inhibitor for sulphydryl dependent proteases (HEI) is a low molecular weight protein. It is antigenic and very soluble, being easily extracted from epidermis in dilute buffers, and this hinders detection by immunofluorescence methods in fresh cut tissue sections. It is feasible that this inhibitor protein is released from epidermis to the underlying dermis and to the blood during diseases of the epidermis initiating the formation of autoantibodies to HEI. In this report we describe a sensitive assay method which confirms the presence of autoantibodies.

The assay is based on a solid phase RIA and is technically simple. The studies with the hyperimmune rabbit sera and the controls indicate the specificity of the assay and that it measures the presence of specific antibodies to the epidermal inhibitor protein.

The antibody titres were low in patients when compared to the titres found in rabbits immunized against the HEI protein. Significantly increased titres were found in the patients who all had widespread dermatitic conditions independent of the underlying primary dermatosis. This indicates that the antibodies are not restricted to any specific disease, but reflect any epidermal destruction which allows liberation of HEI protein. It is possible that these antibodies may be enriched at the sites where HEI is released and they may modify the further development of the disease process. The biological function of the inhibitor in healthy or diseased skin is not known, but it is likely to play a part in regulating the local activity of the epidermal sulphydryl dependent proteases (Hopsu-Havu, Fräki

& Järvinen 1977). The proteases in turn may be active in protein metabolism, keratinisation, inflammation and cell proliferation. By binding the protease inhibitor (HEI), the antibodies destroy its inhibitory capacity, as shown *in vitro* by Järvinen (1978), and thus accentuate the protease functions. The end result may be an exacerbated or delayed course of the disease.

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Measurement of biological ageing in human epidermis

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SUMMARY

Measurements were made of the thickness of the epidermis, the dimensions of keratinocytes and of the surface areas of individual corneocytes in normal human skin from three non-light exposed sites. The numbers of cells in the epidermis and their height decreased with age, whereas the surface area of individual corneocytes tended to increase with increasing age. The changes demonstrated may be responsible for some of the alterations noted clinically in the skin of elderly individuals.

Measurement of tissue ageing is of importance both to the interpretation of the effects of diverse external stimuli and to the study of the effect of dietary and chemical manipulations on the ageing process. Many of the tests devised measure ageing in individual tissues or organ systems and 'test batteries' have been employed to obtain an overall assessment of ageing in the organism as a whole (Comfort, 1979). Tests that measure a complex function such as the 'hand grip strength test' or determine a biochemical parameter summate the effects of disease, cumulated environmental traumata and true biological ageing and cannot in themselves be used to measure the ageing process. Histometric techniques have the advantage that inspection of the tissue allows identification of the effects of disease and exclusion if the effects of ageing alone are to be considered. They have the disadvantage of any invasive technique and in addition are time consuming.

Skin is an ideal organ for the investigation of age dependent changes because of its accessibility. In addition, because of its admixture of tissue types and the different functions that it subserves it is possible to obtain a broad picture of the changes due to ageing.

This study attempts to define age related change in overall epidermal dimensions and in parameters of keratinocyte and corneocyte size.

METHODS

Subjects investigated

Healthy individuals or patients with minor and localized skin disorders who had no systemic disorders, between the ages of 15 and 92, were studied. All individuals gave their consent after full

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explanation of the nature of the study. Most subjects were white caucasian who were normally resident in the South Wales area, but there were also three black subjects who were born in the West Indies and four brown-skinned subjects who came from the Indian subcontinent. Previous studies of epidermal dimensions (Holt & Marks, 1976; Delforno, Holt & Marks 1978; Marks, 1979) have not revealed a consistent or significant sex or racial difference and we have pooled the data for individuals of different race and sex.

Sites investigated

The following usually non-exposed sites were studied: (a) middle of the inner aspect of either upper arm; (b) middle of the flexor aspect of either forearm; (c) middle of outer aspect of either thigh; (d) lower abdomen (middle of either iliac fossa). Not all subjects had all sites investigated.

Tissue sampling

The above sites were biopsied using 4 mm disposable 'punch biopsy' trephines after local anaesthesia had been obtained by injection of 1% lignocaine. The biopsies were fixed in 10% formalin and histologically prepared according to standard techniques. Sections were cut at 6 μm and stained with haematoxylin and eosin. Sections that were not perfectly orientated in the vertical plane were not assessed. Superficial horn cells (corneocytes) were obtained from the same sites by gentle agitation of the skin surface with a Teflon rod within a 'collar' of the same material containing a small amount of phosphate buffered 0·1% Triton X-100. The buffer and its corneocyte content were aspirated and 'smears' prepared on glass microscope slides.

Measurement techniques

Histological sections. Mean epidermal thickness in μm and in numbers of cells between the basement membrane and the beginning of the granular layer in the vertical plane was obtained for each section. The measurements were obtained either by use of a microscope drawing tube and measurement of the projected image or by use of a Quantimet 720 image analysis system. Observations were taken at a minimum of 20 points along the epidermis. From the mean epidermal thickness in μm and cell number (MCN) a theoretical value for the height of viable keratinocytes (mean keratinocyte height, Mkh) was calculated for each specimen (μm/MCN = Mkh). In addition to these measurements the ratio of the length of the basal layer to the length of the base of the granular layer was determined using a map curvimeter on the drawn projected image (B/G ratio). The mean length of epidermal cells was determined with the techniques described above by counting the number of cells along a measured length of basement membrane (mean keratinocyte length, Mkl). The reproducibility, accuracy and limitations of these techniques, as well as the effects of site of sampling and sex on the values obtained, are discussed elsewhere (Marks, 1979).

Corneocytes. The mean corneocyte area (MCA) in μm^2 was also obtained from projection drawings of smears and measurement in the Quantimet 720 image analyser. Between 30 and 50 individual corneocytes were assessed for each smear.

RESULTS

The correlation between the chronological age and the mean keratinocyte height for each subject in the sites examined is depicted in Figs 1, 2 and 3. The coefficients of correlation for the three sites are quite similar (r = 0·5558, 0·506 and 0·568 for arm, abdomen and thigh respectively). The means

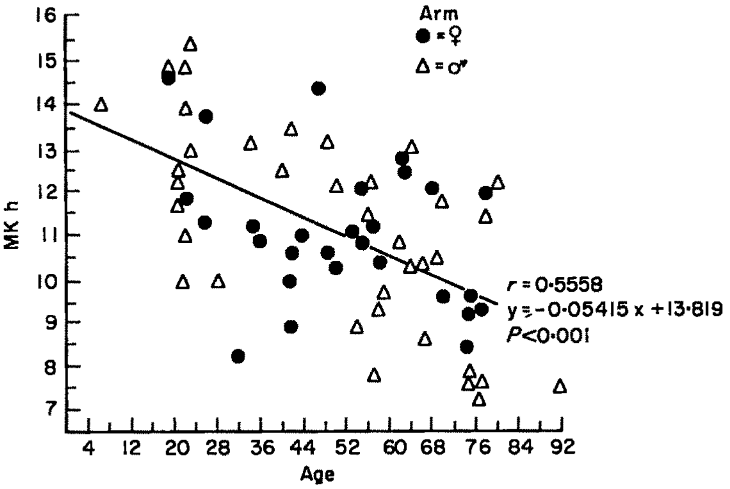

FIGURE 1. Correlation between mean keratinocyte height and age in upper arm site.

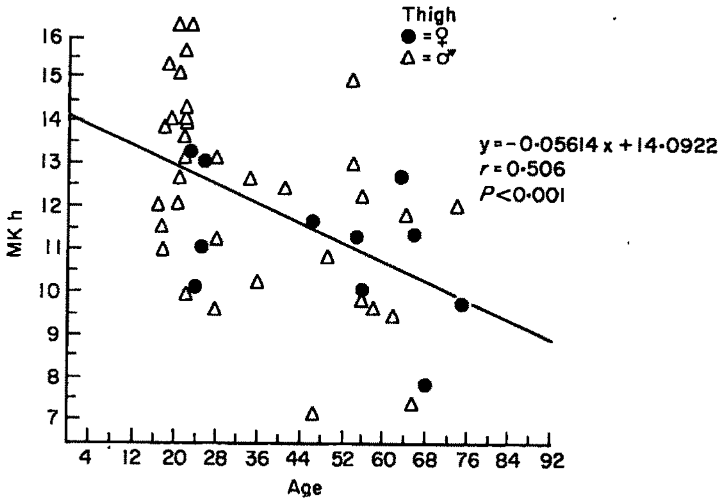

FIGURE 2. Correlation between mean keratinocyte height and age in thigh site.

and standard deviations for B/G ratio, mean epidermal thickness in absolute terms (μm), mean epidermal thickness in cell numbers (MCN) and mean keratinocyte length for the various sites and for arbitrary 15-year periods are given in Table 1. It is observed that there is a decrease in MCN for the arm and thigh sites of approximately one cell, but this is less marked on the abdomen. The drop in Mkh is between 10 and 20% at the three sites, being most marked in the upper arm. There is some tendency for Mkl to increase with age, but this is less regular.

The correlation for mean corneocyte areas with age in the upper arm is given in Fig. 4 and the means and standard deviations for the various sites and for the same arbitrary 15-year periods are given in Table 2. The correlations between age and corneocyte area for the thigh and abdomen are weaker than for the arm, but the means of the arbitrarily chosen age divisions indicate that there is a tendency for corneocyte area to increase with age.

TABLE 1. Means, standard deviations and number of observations of basal cell layer length/granular cell layer length ratio (B/G), mean epidermal thickness in cell numbers (MCN), mean keratinocyte height in μm (Mkh) and mean keratinocyte length in μm (Mkl) in four age groups at three sites for both sexes

Age Group		Upper arm				Thigh				Abdomen			
		B/G	MCN	Mkh	Mkl	B/G	MCN	Mkh	Mkl	B/G	MCN	Mkh	Mkl
15–34	Mean	1·07	4·05	11·96	6·28	1·10	4·64	11·95	6·71	1·19	4·05	12·90	7·18
	s.d.	0·13	0·58	2·04	0·72	0·14	0·50	2·18	0·93	0.21	0·55	1·95	0·86
	No.	17	19	19	19	24	32	32	32	14	21	21	21
35–54	Mean	1·06	3·84	11·43	6·46	1·09	3·93	9·97	6·4	1·14	3·75	10·99	6·93
	s.d.	0·10	0·62	1·85	0·59	0·09	0·68	1·83	0·81	0·18	0·36	1·85	0·82
	No.	10	14	14	14	10	10	10	10	8	8	8	8
55–74	Mean	1·04	3·64	10.78	6·87	1·06	3·89	10·62	6·76	1·06	3·97	9·73	6·27
	s.d.	0·04	0·71	1·51	0·58	0·08	0·51	1·83	0·87	0.12	0.41	1·64	0·43
	No.	11	18	18	18	15	18	18	18	6	6	6	6
75+	Mean	1·07	3·03	9·73	7·09	1·05	3·30	10·73	7·23	1·26	3·70	11·17	7·13
	s.d.	0·08	0·33	2·26	0·69		0·82	2·55	0·70	0·30	0·29	3·36	1·00
	No.	8	12	12	12	2	3	3	3	4	4	4	4

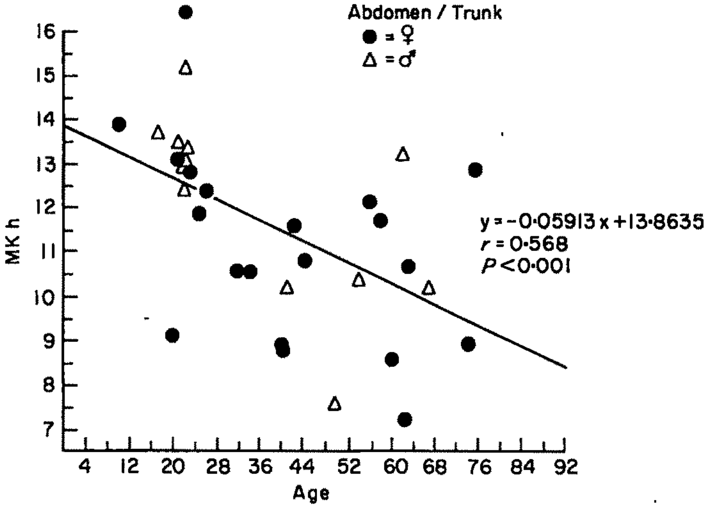

FIGURE 3. Correlation between mean keratinocyte height and age in lower abdomen site.

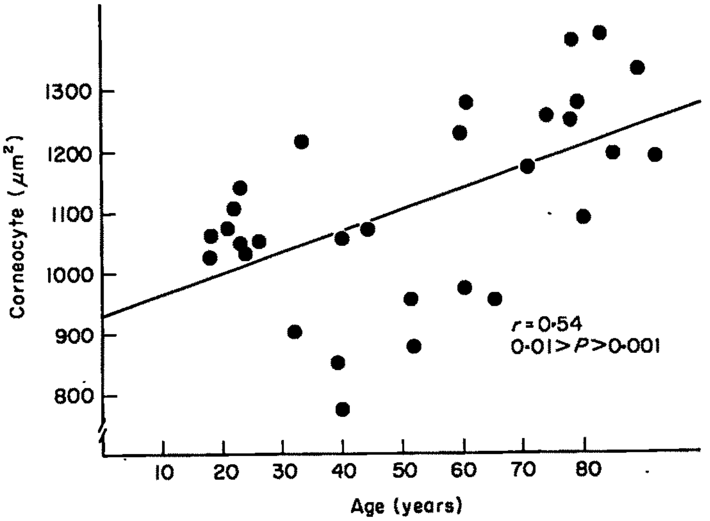

FIGURE 4. Correlation between mean corneocyte area and age in upper arm site.

TABLE 2. Means, standard deviations and number of observations of mean corneocyte area in μm^2 in four age groups at three sites for both sexes

Age group		Upper arm	Thigh	Abdomen
15–34	Mean	1077·9	1160·2	1041·6
	s.d.	120·9	180·6	118·5
	No.	14	25	9
35–54	Mean	1008·3	1148·1	1186·7
	s.d.	185·6	131·9	222·9
	No.	10	12	9
55–74	Mean	1153·2	1247.1	1053.8
	s.d.	183·1	127·6	116·3
	No.	12	15	5
75+	Mean	1239·9	1200·9	1258·1
	s.d.	100·8		192·6
	No.	7	2	5

COMMENT

Certain of the changes that take place in skin with age have been studied previously. For example, wound healing of skin has been found to be age dependent (Billingham & Russell, 1956), but its measurement is difficult and investigation in man impractical. Skin thickness also decreases with age (Black, 1969) and other dermal physical properties have been found to be age dependent (Elden, 1965; Parot & Bouliere, 1967). These measurements depend on several tissue components and are more likely to be influenced by environmental circumstances such as nutritional status and previous solar exposure.

The number of melanocytes in the epidermis has been found to decrease with age, though whether this is relative to the number of surrounding basal cells or in absolute terms is uncertain (Fitzpatrick, Szabo & Mitchell, 1964). Snell & Bischitz (1963) found that melanocytes decreased by 11% per decade postnatally. Melanogenesis in the skin also decreases in the elderly, though apparently the rate of pigment formation in the retinal pigment cells is maintained. Fitzpatrick *et al.* (1964) also stated that melanocytes increase in size with age and implied that this fitted Minot's finding that ageing cells increase in size (Minot, 1908).

The time to initiation of fibroblast outgrowth from small skin explants *in vitro* has also been found to decrease with the age of the donor, although no differences were noted in epithelial outgrowth (Waters & Walford, 1970).

The findings presented here suggest that there is a continuous decrease in population size of the epidermis with increasing age and that there is a tendency for the constituent cells to decrease in vertical height. With the decrease in mean keratinocyte height there may be a small increase in mean keratinocyte length, i.e. the cells tend to become shorter and fatter. However, if the theoretical volumes of the keratinocytes (height × length^2) are plotted as a function of age there is still a tendency for there to be a decreased 'mean keratinocyte volume' with age, though the correlation is poor. Preliminary studies with suspensions of epidermal cells using the channeliser function of the Coulter counter to obtain 'modal volume' indicate that the volume changes with age found histometrically are also present in cell suspensions (Marks, unpublished observations).

Surprisingly, there is little information on the change in rates of epidermal cell production with increasing age. Thuringer & Katzberg (1959) reported that the number of mitoses found in human abdominal skin decreased from childhood to the fifth decade and then remained static. Another group using tritiated thymidine autoradiography found no relationship between age and epidermal cell renewal time (Epstein and Maibach, 1965) (Fig. 3). Cameron (1972) found a decreasing epidermal cell renewal time in the mouse. It has been found that stratum corneum renewal time is increased in old age (Baker & Blair, 1967), also suggesting that the rate of epidermopoiesis is decreased.

It is of interest that there is some increase in surface area of corneocytes with increasing age. This has been noted previously (Plewig, 1970; Grove, 1979). It is possible that the increased surface areas observed reflect an increased stratum corneum transit time with increased flattening of individual corneocytes.

The skin of the elderly feels 'dry' and slightly rougher than in maturity. It is possible that the epidermal changes described are at least partially responsible for these alterations.

ACKNOWLEDGMENT

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British Journal of Dermatology (1981) **104**, 635.

Studies on the plasma membrane of normal and psoriatic keratinocytes. 3. Uptake of labelled sugars and their incorporation into glycoconjugates

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SUMMARY

We report the uptake of four labelled sugars by keratinocytes isolated from normal epidermis, psoriatic 'uninvolved' skin and psoriatic lesions. Our findings include the following:

(1) The rate of uptake of all sugars by the psoriatic lesion is increased.

(2) This abnormally high uptake diminishes dramatically during 22 h incubation *in vitro*.

(3) There is a striking abnormality in the metabolism of fucose by psoriatic keratinocytes; our data suggest an increased rate of incorporation of fucose into glycoconjugates.

We have already described morphological changes (Gommans *et al.*, 1979a) and functional disturbances (Gommans *et al.*, 1979b) of the plasma membrane in psoriatic keratinocytes. The remaining papers in this series will deal with the glycoconjugates (glycoprotein, glycolipid and proteoglycans) which are essential components of the membrane and its receptors.

In this communication we report the uptake of labelled sugars by isolated keratinocytes and their incorporation into glycoconjugates. In particular, we have asked two questions. First, to what extent can the known membrane abnormalities in psoriasis (Mahrle & Orfanos, 1977) be characterized in terms of a specific biochemical defect? Second, if such a defect exists, is this also manifest in the clinically uninvolved skin of the psoriatic patient?

MATERIALS AND METHODS

Materials

L-(1-^{14}C)fucose (61 Ci/mol), N-acetyl-D-(1-^{14}C) glucosamine (59 Ci/mol), D-(1-^{14}C) galactose (60·7 Ci/mol), D-(1-^{14}C) mannose (59 Ci/mol), L-(6-^{3}H)fucose (17·1 Ci/mmol) and D-(6-^{3}H)glucosamine hydrochloride (38 Ci/mmol) were obtained from the Radiochemical Centre, Amersham. All other chemicals were as described previously (Gommans *et al.*, 1979a).

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The selection of healthy volunteers and psoriatic patients, the method of biopsy and the preparation of isolated keratinocytes have already been reported (Gommans *et al.*, 1979a).

Labelling procedures

Keratinocytes (usually 1–2 × 10^6) from healthy controls, psoriatic lesions or psoriatic 'uninvolved' skin were suspended in 0·5 ml TC 199 medium supplemented with 20% calf serum and containing 200 units/ml penicillin and 100 μg/ml streptomycin sulphate. The appropriate radioactive sugar was added (1 μCi/ml for ^{14}C-labelled sugars or 10 μCi/ml for ^{3}H-labelled sugars) and the suspension was incubated at 37°C. At the end of the appropriate incubation period, the cells were pelleted by centrifugation (400 *g*, 5 min) and washed three times with 0·5 ml portions of TC 199 medium without serum, the washings being discarded. The washed cells were finally extracted three times with 0·5 ml portions of ice-cold trichloroacetic acid (5% w/v), with centrifugation at 2300 *g* for 10 min. Radioactivity was measured in the pellet after solubilization in 1% NH_3 ('TCA-insoluble sugar') and in the pooled extracts ('TCA-soluble sugar').

Three time-schemes were employed:

(1) A single labelling period of 3 or 4 h (t = 3 or 4).
(2) A single labelling period of 22 h (t = 22).
(3) Pre-incubation in TC 199 plus serum for 22 h followed by a labelling period of 3 or 4 h (t = 22/3 or 22/4).

Scintillation counting

All samples were counted in an Isocap 300 (Searle Ned. B.V., Uithoorn, NL) after addition of 10 ml 'aquasol' (NEN, Inc., Boston, Mass.). External standard ratios were used for quench correction.

RESULTS AND DISCUSSION

Uptake and distribution of radioactivity

Our results are summarized in Fig. 1. In each group of experiments we have indicated the mean total radioactivity of the keratinocytes, together with the distribution into TCA-soluble material (free sugar, sugar phosphates, UDP-sugars, etc.) and TCA-insoluble material (glycoprotein, glycolipid, proteoglycans).

The effects of different concentrations of 'cold' sugar in the incubation medium are seen by comparing the results using ^{3}H-labelled fucose and N-acetylglucosamine with the corresponding experiments in which ^{14}C-labelled sugars were employed. It is clear that the use of the very high specific activity ^{3}H-label (i.e. the addition of less 'cold' sugar to the medium) results not only in a higher absolute level of radioactivity taken up, but also in a rather higher percentage of the total activity appearing in the TCA-insoluble fraction. This latter observation implies that the synthesis of glycoconjugates in the Golgi apparatus may be the rate-limiting step in the overall pathway.

Assumptions and theoretical model

The interpretation of data such as these necessitates a theoretical model which must be based on certain simplifying assumptions. Here we may reasonably assume that the label, after entry into the cell, passes through two consecutive metabolic pools, namely the unconjugated sugars (TCA-soluble pool) and the conjugated sugars (TCA-insoluble pool), as shown in Fig. 2. If the cell is in metabolic equilibrium, the pool sizes are constant and $V_1 = V_2 = V_3$ ('turnover rate').

These two parameters (pool size and turnover rate) may in principle be evaluated by the appropriate choice of labelling period.

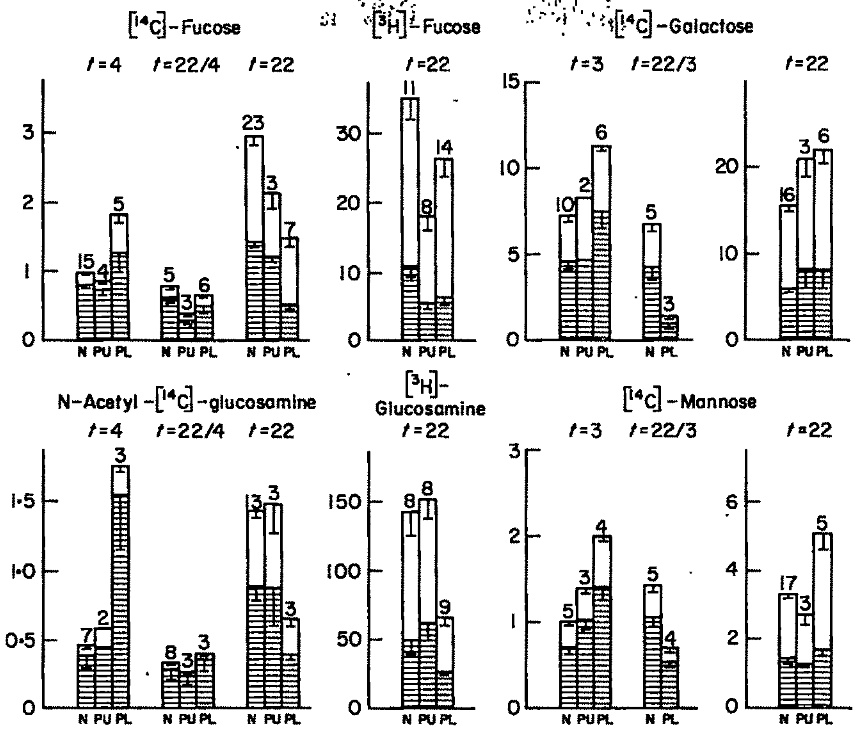

FIGURE 1. Radioactivity (c.p.m. per 10^3 cells) in the TCA-soluble fractions (shaded bars) and the TCA-insoluble fractions (open bars). The figure above each bar indicates the number of specimens measured, and the vertical lines represent the s.e.mean. Time-schedules are described in the text ('labelling procedures'). Abbreviations are: N = normal epidermis, PU = psoriatic uninvolved, PL = psoriatic lesion.

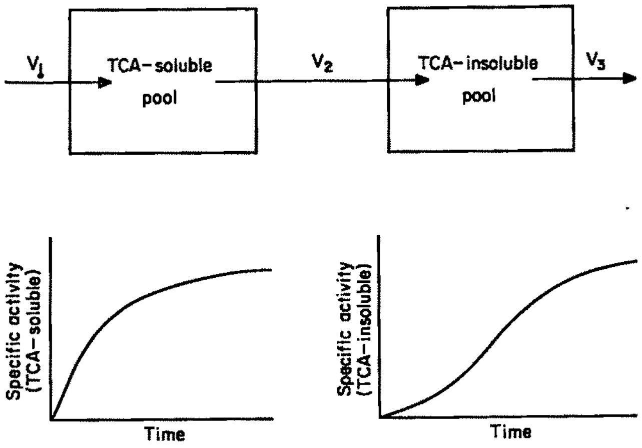
V_1
TCA-soluble pool
V_2
TCA-insoluble pool
V_3
Specific activity (TCA-soluble)
Time
Specific activity (TCA-insoluble)
Time

FIGURE 2. Idealized model for transit of the labelled sugar through the cell. The implicit assumptions are described in the text. Note that V_1, V_2 and V_3 represent bulk transport rates and not movement of radioactivity.

Turnover rate. For very short labelling periods, the label leaving a pool is small compared with that entering; the initial rate at which radioactivity accumulates is therefore governed only by the turnover rate and is independent of pool size. For the TCA-soluble pool, the calculation is simplified by the fact that the precursor (i.e. the extracellular sugar) remains at constant specific activity. The initial rate of increase will therefore be linear, and the slope will be proportional to V_1. In the case of the TCA-insoluble pool, however, the situation is more complex; here we must also take into account the 'average' specific activity of the material entering the pool in order to compare bulk-flow rates (V_2) between different experiments. This has been done for 'short' labelling periods by expressing the results as the ratio of the activity of the TCA-insoluble pool to that of the TCA-soluble pool (Fig. 3).

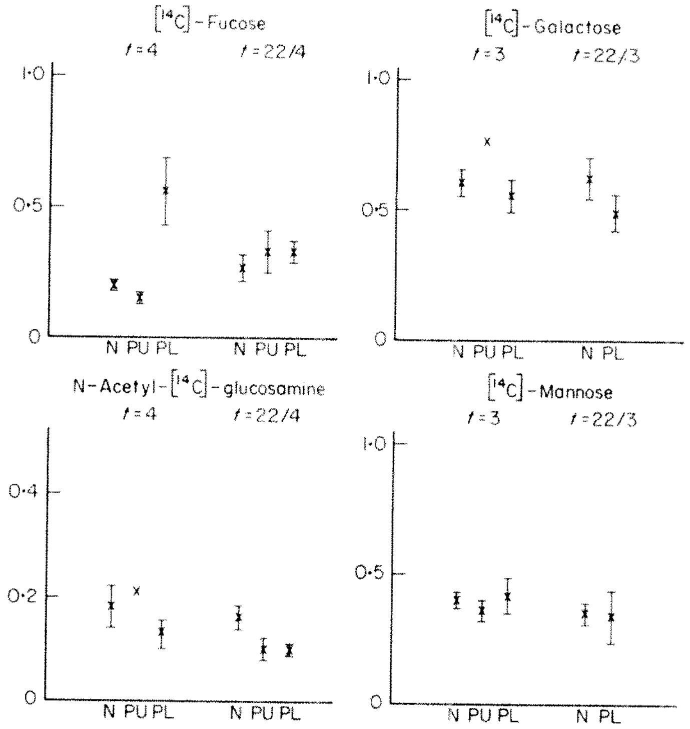

FIGURE 3. Ratios of TCA-insoluble to TCA-soluble activity. Abbreviations are: N = normal epidermis, PU = psoriatic uninvolved, PL = psoriatic lesion.

Pool sizes. After a sufficiently long labelling period, a dynamic equilibrium is reached in which the specific activities of the pools are equal and constant. At this point the total activity of each pool is a direct measure of its size.

The practical measurement of flow-rates and pool sizes thus depends on selecting optimum values for the labelling periods. The lower limit for the 'short' period is set by the minimum cpm which can be accurately determined; this was 3–4 h for routine measurements. Preliminary experiments, using normal human keratinocytes, confirmed that the net uptake of radioactivity was reasonably linear during this time. The choice of the 'equilibration' period is more difficult because protracted periods of maintenance *in vitro* may well lead to gross metabolic changes during the experiment. Preliminary investigations indicated that the total radioactivity taken up by normal keratinocytes increases little after 22 h; the possibility of metabolic alteration during this time is excluded by the observation that pre-incubation for 22 h followed by a 'short' labelling period (Fig. 1, t = 22/3 or 22/4) yield results similar to those from fresh cells (t = 3 or 4). This was not true, however, for psoriatic keratinocytes (see below).

The TCA-soluble pool (unconjugated sugars)

Application of this model to the data presented in Fig. 1 yields two clear-cut conclusions regarding the entry-rates of sugars into the TCA-soluble pool. First, using freshly-trypsinized preparations of keratinocytes, it is seen that the rates of entry of all four sugars into cells derived from psoriatic lesions are very much higher than into normal cells (t = 3 or 4). Secondly, the rates of entry of sugars into psoriatic keratinocytes, in contrast to normal cells, decline dramatically after 22 h pre-incubation *in vitro* (t = 22/3 or 22/4). This latter phenomenon is apparent both in keratinocytes derived from lesions and from uninvolved skin, but is particularly striking in the case of the lesions.

Surprisingly, the radioactivity present in the TCA-soluble pool after a 'long' labelling period (t = 22) in cells derived from psoriatic lesions is not increased over the level found after a 'short' period; indeed, in the cases of fucose and N-acetylglucosamine it is significantly less. This confirms our previous conclusion that the metabolic parameters of psoriatic keratinocytes change rapidly *in vitro*, and prohibits quantitative statements regarding pool sizes.

The TCA-insoluble pool (glycoconjugates)

The rate of entry of a sugar into the TCA-insoluble pool (i.e. the rate of synthesis of glycoconjugates) is indicated by the ratio of activity (TCA-insoluble): activity (TCA-soluble). These ratios have been calculated from the data of Fig. 1 and are presented in Fig. 3. A striking and apparently specific abnormality is seen in the experiments using fucose. Comparing freshly trypsinized keratinocytes from psoriatic lesions with those from healthy controls, it is evident that the rate of incorporation of fucose into glycoconjugates is about three times greater than normal. This high value, however, returns to a figure approaching normal after 22 h pre-incubation *in vitro* (t = 22/4). Curiously, keratinocytes derived from psoriatic uninvolved skin behave in precisely the opposite manner; fresh cells have a somewhat low rate of incorporation of fucose, which rises significantly after pre-incubation.

Again the evaluation of pool sizes is hindered by our observation that the metabolism of psoriatic keratinocytes is changing during the 'long' labelling period. It is evident that the pattern of sugar incorporation into glycoconjugates (Fig. 1, t = 22) is grossly abnormal in the psoriatic cells, but our data give no indication of whether this conclusion may be extrapolated to the situation *in vitro.*

General comments

Three conclusions have been drawn from the data in Fig. 1. These are firstly, that keratinocytes from psoriatic lesions take up all sugars more rapidly from the medium, secondly, that this abnormally high rate of uptake falls dramatically during incubation *in vitro*, and finally, that there seems to be a specific abnormality in the incorporation of fucose into glycoconjugates in psoriatic cells.

Such conclusions are, of course, dependent on the validity of the assumptions inherent in the model used to interpret the data. We have already pointed out that comparisons of pool sizes might be misleading because the rate of uptake of sugars by the psoriatic cells does not remain constant during the experiment. Other potential inaccuracies in our assumptions include:

(1) *De novo* synthesis of sugar, leading to dilution of the labelled material entering the cell.

(2) Recycling of sugar via intracellular breakdown of glycoconjugates.

(3) Metabolic utilization of the labelled sugar via alternative pathways, for example conversion to other sugars.

We obviously cannot exclude any of these possibilities on the basis of the data presented. Indeed, attempts to calculate absolute pool sizes from the radioactivity after 22 h and the specific activity of the added sugar yield results at least an order of magnitude less than values generally reported for chemical assays (Yurchenko, Ceccarini & Atkinson, 1978), implying considerable levels of *de novo* synthesis or recycling. However, these limitations do not necessarily invalidate qualitative statements regarding flow-rates. At the worst, alternative explanations are introduced; in particular, the apparently increased incorporation of fucose into glycoconjugates of psoriatic lesions could instead reflect a diminished *de novo* synthesis of fucose (and hence a 'hotter' TCA-soluble pool) in psoriatic cells.

The striking metabolic changes observed in psoriatic keratinocytes after 22 h incubation *in vitro* are perhaps not surprising. The situation seems to parallel that occurring *in vivo*, where a transit time of only 3 days (Porter & Shuster, 1968) suggests an enormous acceleration of the various processes which are collectively termed 'keratinization'. These processes include progressive losses of enzymatic activities and of membrane functions in addition to the oxidation of prekeratin to mature keratin. Our present results imply that in cells from the psoriatic lesion the transport function diminishes very rapidly indeed; the relatively small changes in the percentage incorporation of three sugars into glycoconjugates after 22 h pre-incubation indicate that the Golgi apparatus and the endoplasmic reticulum maintain their integrity during this period, i.e. that 'cell death' has not occurred.

The findings regarding fucose metabolism are of greater interest in that they are compatible with a specific membrane defect. We are currently engaged in a more detailed study of the composition of the TCA precipitate (glycoprotein, glycolipid and proteoglycans) and also in the investigation of cell-surface material removed by trypsin in order to elucidate this abnormality more clearly.

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Lectin binding studies on suspensions of isolated epidermal keratinocytes

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SUMMARY

The sugar composition of the surface of isolated human keratinocytes has been investigated using lectins. A method is described in which the FITC-labelled lectin is displaced with competing sugar prior to measurement. This avoids errors due to aspecific binding and utilizes the minimum biological sample. Evidence is presented which indicates that little cell surface glycoconjugate is lost during the preparation of the keratinocyte suspension from human epidermal slices using the technique described here. Values for keratinocytes are similar to those reported for fibroblast lines, being much higher than those found using erythrocytes or lymphocytes.

The epithelium of human skin is a complex structure, consisting of several distinct layers of cells. Proliferation occurs exclusively in the innermost (basal) layer. Certain cells move outward and lose their mitotic ability, this stage being characterized by extensive synthesis of pre-keratin (spinous layer). The uppermost spinous cells subsequently undergo extensive morphological and biochemical changes (keratinization) to leave what is essentially a flake of mature keratin (the corneocyte). It is this last layer which provides the chemical barrier between the body and the environment, and which may therefore be regarded as the *raison d'être* of the epidermis.

Clearly the ability of such a complex system to function in the face of environmental fluctuations requires active coordination of cell division, differentiation and keratinization. Surprisingly, however, little is known about the homeostatic processes involved, beyond recognition that certain pharmacological mediators influence the equilibria *in vivo* or in culture. Most or all of these mediators act via cell-surface receptors which depend for their function on the presence of specific sugar moieties.

Lectins, which are proteins or glycoproteins originally isolated from plant seeds, are characterized by their ability to bind sugar residues. Their value for the investigation of membrane-bound carbohydrate is now well documented (Hughes, 1976). It seems likely, therefore, that any changes in the distribution of surface receptors which accompany the normal processes of differentiation or pathological situations such as psoriasis may be evaluated in terms of alterations in the lectin-binding properties of the keratinocyte. In this report we describe the binding of five different lectins to normal human keratinocytes.

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MATERIALS AND METHODS

Chemicals

α-Methyl mannosidase, N-acetyl-D-galactosamine, D-galactose, L-fucose, N-acetyl-D-glucosamine, trypsin (grade III), ribonuclease (bovine pancreas, 5 × recrystallized) and sialidase (type VI, *Clostridium perfringes*) were obtained from the Sigma Chemical Co., Missouri. Medium (TC 199) was supplied by Difco (Detroit), penicillin and streptomycin sulphate by Mycofarm, Delft, and calf serum by the Animal Laboratory of this University.

The various FITC-labelled lectins (Table 1) were purchased from Pharmindustry, Clichy, France. The protein content of each batch of lectin was determined by measurement of optical densities at 278 and 492 nm as described by Monsigny & Obrenovitch (1979). All other chemicals were of Analytic Reagent quality and were obtained from Merck (Darmstadt, Germany).

Skin samples and preparation of cells

This has been described in detail in a previous communication (Gommans *et al.*, 1979a). In brief, samples of split-thickness skin (about 1 cm^2, 0·1 mm thick) were removed from paid volunteers using a Castroviejo keratotome. After incubation with trypsin and dithioerythritol (30 min, 37°C) the epidermis was peeled from the dermis and the keratinocytes dispersed by agitation in TC 199 medium containing 20% calf serum. After treatment with ribonuclease and filtration (50 μm pore size) the cells were pelleted, resuspended in an appropriate volume of medium and counted. Typically 3–6 × 10^6 keratinocytes could be obtained from each specimen.

Determination of binding sites and association constants

All manipulations using ConA or UeA were carried out in 15 mM tris-buffered isotonic saline containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$ and 1 g/l bovine serum albumin (TBSMA). The experiments using HPA, PNA or WGA employed phosphate-buffered saline containing 1 g/l bovine serum albumin (PBSA).

Freshly-prepared keratinocytes were washed twice in TBSMA or PBSA with centrifuging at 150 *g* and distributed into six 'Eppendorf' polypropylene tubes. Each aliquot contained 1–3 × 10^5 cells, a quantity which gave saturation within the range of lectin concentration used. FITC-lectin, dissolved in TBSMA or PBSA, was added to each tube to yield a total volume of 200 μl and a final lectin concentration within the range indicated in Table 1. Blanks (cells omitted) were set up corresponding to the highest and lowest lectin concentrations.

After 1 h at 20°C the cells were pelleted, washed twice, and extracted with 500 μl of the appropriate eluting agent (Table 1) for 30 min. The cells were again pelleted, and the fluorescence of the extract determined for specific bindings ('Fluorispec', Baird Atomic; $\lambda_{EX} = 488$, $\lambda_{EM} = 522$ nm). 500 μl of buffer was added to the remaining pellet, and after ultrasonic treatment (MK2, MSE Scientific Instruments Ltd, England) the fluorescence of the contents was determined for aspecific binding. A standard of 1–5 μg FITC-lectin in the appropriate eluting agent was employed for calibration. Cell-bound lectin was calculated by subtracting the appropriate blank, and free lectin calculated by subtraction of total bound lectin from the orginal quantity of lectin employed.

After incubation some batches were checked for possible internalization of the lectin with a fluorescence microscope (Leitz Ortholux).

A Scatchard plot (Fig. 1) was used to determine the number of specific binding sites per cell and the association constant according to the equation:

TABLE 1. A summary of the properties of the FITC labelled lectins and the experimental conditions employed. The measurement of FITC/protein ratios is described in the text

Lectin	Abbreviation in text	mol.wt.	Average FITC/protein ratio	Source	Sugar specificity	Range of concentration (mg/l) employed	Eluting agent
Concanavalin A	ConA	110,000	0·25	Jack bean: *Canavalia ensiformis*	Glucose Mannose	25–375	0·15 M Methyl mannoside
Helix Pomatia agglutinin	HPA	79,000	0·20	Snail: *Helix pomatia*	N-acetyl-D-galactosamine	25–275	0·06 M N-Acetyl-D-galactosamine
Peanut agglutinin	PNA	108,000	0·85	*Arachis hypogea*	β-D-galactose in a non-reducing terminal position	5–75	0·15 M D-Galactose
Ulex europeus agglutinin	UeA	46,000	0·50	Gorse seeds (glycoprotein fraction I)	L-Fucose	25–375	1 M Fucose
Wheat germ agglutinin	WGA	36,000	0·25	Wheat germ: *Triticum vulgare*	N-acetyl-glucosamine (sialic acid)	20–300	0·3 M N-Acetyl glucosamine

$$\frac{B}{F} = (B_{max} \times K_{ass}) - (B \times K_{ass})$$

where

B = bound lectin (M);
F = free lectin (M);
B_{max} = maximal binding (M) which is equal to the binding site concentration;
K_{ass} = association constant (M^{-1}).

Regression lines were calculated using a Texas Instrument SR-51-II and experiments yielding a correlation coefficient of $< |0{\cdot}90|$ were discarded.

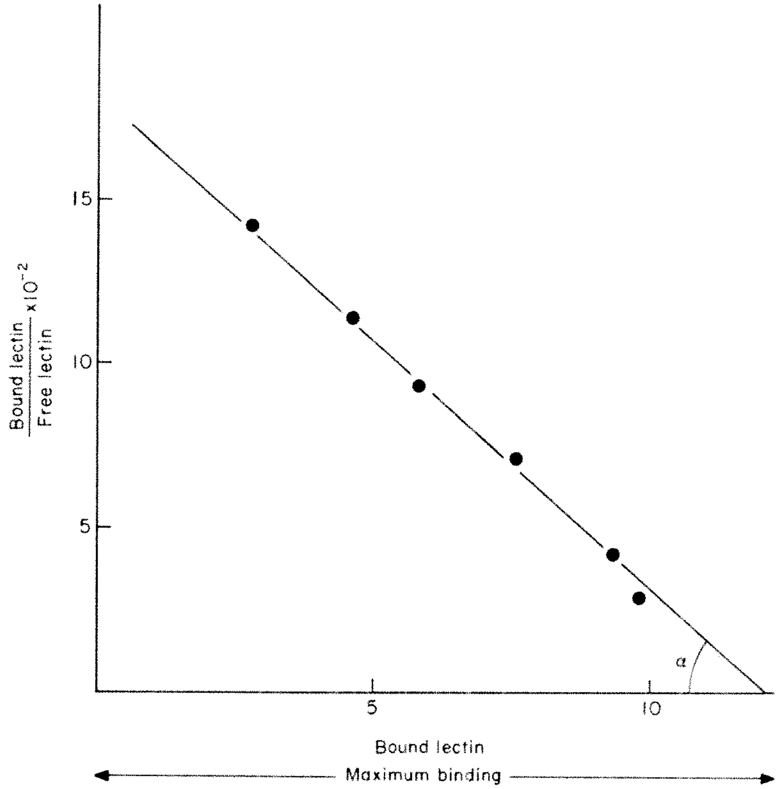

FIGURE 1. A typical Scatchard plot for ConA binding to keratinocytes. Bound and free lectin are expressed as μg/ml. In this experiment the number of cells employed was $2{\cdot}03 \times 10^5$ per tube.

Maintenance of keratinocytes in vitro

In certain investigations keratinocytes were maintained *in vitro* for various periods prior to lectin binding studies. Two alternative techniques were employed. In some experiments the cells, suspended in TC 199 medium supplemented with 20% calf serum, were allowed to attach to the surface of glass vessels during the incubation period; they were subsequently dislodged by gentle friction with a rubber policeman. In other experiments aliquots of the cell suspension were placed in Eppendorf polypropylene tubes and agitated on a reciprocal mixer adjusted to the minimum amplitude necessary to prevent settling. In all cases the cells were afterwards washed twice in the appropriate buffer and the lectin binding measured in the usual way.

Incubation of isolated keratinocytes with trypsin or sialidase

The effect of trypsin was investigated by incubating 1–2 × 10^6 cells with 0·025% w/v trypsin and 0·3% w/v dithioerythritol in an Eppendorf polypropylene tube (total volume 1 ml) agitated on a reciprocal shaker at 37°C. In certain experiments (Fig. 2) the basal medium was TC 199 and the incubation period was 30 min; viability after incubation, as measured by the exclusion of trypan blue, was 80–90%. For other experiments (Table 4), phosphate-buffered saline (pH 7·3) was employed and the incubation period was 10 min; this resulted in a viability of only about 60%.

Incubation with sialidase employed 0·5 units/ml of the enzyme. The basal medium was phosphate-buffered saline at a pH of 6·5 and the incubation period 10 min. Conditions were in other respects as for trypsin. Viability was again about 60%.

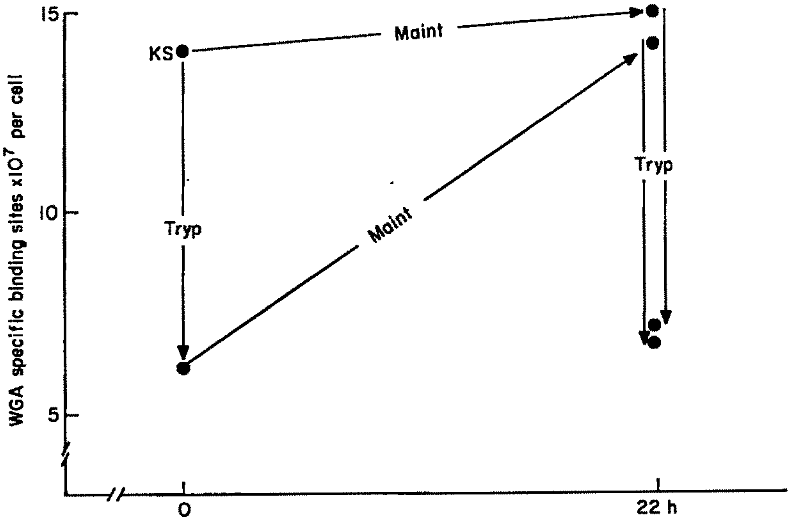

FIGURE 2. Results of various protocols illustrating the reversibility of receptor cleavage by trypsin and recovery during maintenance *in vitro*: KS = freshly prepared keratinocytes; tryp = exposure to trypsin (no serum, 37°C, 30 min); maint = incubation under suspension conditions (TC 199 + 20% serum, 37°C, 22 h).

RESULTS

Specific binding of all five lectins to normal human keratinocytes was observed. No fluorescence of the nuclear membrane, a uniform fluorescence on the cell surface and a fluorescent halo were observed, indicating that internalization of the lectins had not occurred. In general the Scatchard plots were reasonably linear (Fig. 1); values for the calculated number of binding sites and for the association constants are listed in Table 2. With the exception of HPA the proportion of aspecifically-bound lectin was rather low (less than 20% of the total bound lectin). In the case of UeA the aspecific binding was proportional to the concentration of lectin over the observed range; thus no values for aspecific binding sites could be calculated.

The effect of maintenance of the isolated keratinocytes *in vitro* prior to binding measurements is shown in Table 3. It is clear that incubation under conditions such that attachment to the substratum is prohibited has relatively little effect. However, attachment to the surface of a glass culture vessel results in a striking increase in the number of specific binding sites; in the case of WGA this was nearly a twenty-fold increase over the freshly-prepared cells. Measurements after longer periods

TABLE 2. Binding sites (± s.d.) per cell and association constants (± s.d.) for the individual lectins. All cell suspensions were derived from whole epidermis; morphology indicated these preparations to contain an average of about 20% basal and 3.4% 'keratinized' cells, the remainder being designated 'squamous'

Lectin	Nature of binding	Number of specimens	Binding sites $\times 10^7$	Association constant $\times 10^6$
ConA	Specific	53	6·6 ± 2·5	1·71 ± 0·40
	Aspecific	43	1·13 ± 0·35	3·7 ± 1·6
HPA	Specific	11	0·89 ± 0·19	1·81 ± 0·59
	Aspecific	11	0·82 ± 0·39	0·51 ± 0·20
PNA	Specific	11	1·00 ± 0·33	7·1 ± 2·1
	Aspecific	10	0·19 ± 0·08	5·9 ± 2·2
UeA	Specific	8	13·8 ± 3·4	0·13 ± 0·03
	Aspecific			
WGA	Specific	27	14·0 ± 4·6	0·78 ± 0·23
	Aspecific	26	1·87 ± 0·87	0·63 ± 0·21

TABLE 3. Effect of 22 h maintenance *in vitro* on the lectin binding of keratinocytes. Figures are numbers of binding sites per cell ± s.d. Numbers of experiments are in parenthesis

Lectin	Fresh keratinocytes	22 h Maintenance in suspension	22 h Maintenance with attachment
ConA	6.6 ± 2·5 (53)	10·4 ± 6·7 (4)	51·1 ± 24·4 (10)
WGA	14·0 ± 4·6 (27)	15·0 ± 4·6 (14)	264 ± 91 (4)

TABLE 4. Effect of incubating keratinocyte suspensions with trypsin or sialidase. Conditions are described in the text. The control value is taken as 100%

Lectin	Trypsin (%)	Sialidase (%)
ConA	38	115
HPA	58	124
PNA	15	145
UeA	75	166
WGA	36	56

of attachment yielded a mean value of $109 \pm 32 \times 10^7$ binding sites per cell (five experiments) after 46 h and a single value of 424×10^7 binding sites per cell after 6 days *in vitro* for ConA.

The results of incubating keratinocyte suspensions with trypsin or sialidase are summarized in Table 4. The effect of trypsin, as might be expected, is a marked reduction in the binding of all lectins. Following incubation with sialidase, the binding of ConA, HPA, PNA and UeA increase significantly; this is in accord with the concept that removal of sialic acid will reduce steric hindrance and render non-terminal sugars more 'accessible'. Due to possible protease activity in the sialidase preparations this increase may not be optimal. The reduction of binding sites for WGA confirms the report of Boldt *et al.* (1977) that this lectin binds specifically to sialic acid as well as to N-acetyl glucosamine. In certain experiments, incubation with trypsin was followed by 22 h maintenance in suspension. The results of various protocols are illustrated in Fig. 2. It is seen that the loss of surface glycoconjugates is entirely reversible, recovery being complete within 22 h.

DISCUSSION

The techniques described here provide a sensitive, convenient and reproducible method of determining lectin binding sites on epidermal keratinocytes. The advantages of fluorescent labelling over radioactive labelling have already been discussed (Monsigny & Obrenovitch, 1979). In addition the displacement of specifically bound lectin by competing sugar prior to measurement obviates interference from non-specific binding; the alternative technique commonly employed of using a separate 'blank' composed of lectin plus cells in the presence of the competing sugar obviously doubles the number of cells required, a serious problem when dealing with human biopsy material. A theoretical disadvantage of our method is that some specifically bound lectin is lost in the washes, and could result in a low value for apparent binding sites. However, preliminary measurements showed that for no lectin does this loss exceed 10% of the bound material.

Enzyme kinetics and receptor binding kinetics show many common features. The Scatchard plot is a modified Michaelis-Menton equation and therefore three assumptions are implicit; (1) the reaction is at equilibrium and reversible, (2) the lectin concentration is markedly greater than the binding concentration and (3) no cooperative effects between the binding sites occur. In the present study these requirements seem to be fulfilled. The range of lectin concentrations employed (selected after preliminary experiments) reached about seven times the $1/K_{ass}$ values, and no more than about 10% of the total lectin was bound to the cells. Linearity of the plots indicated an absence of cooperative effects, and also confirmed that the specifically bound lectin was attached to the sugar moiety in a relatively uniform configuration. This does not, of course, imply that the individual sugars were present in similar oligosaccharide sequences.

A more serious possibility is the loss of surface glycoprotein during the trypsinization procedure used to isolate keratinocytes. It should be emphasized, however, that in our technique (Gommans *et al.*, 1979a), and in contrast to methods described elsewhere, the separated epidermis is removed from the trypsin solution into medium containing serum *before* mechanical disruption; thus the surface of the isolated cells is never exposed directly to the enzyme. Deliberate subsequent exposure of the cells to trypsin (Fig. 2) does indeed cause serious loss. The data illustrated in Fig. 2 suggest strongly that the surface glycoprotein levels in the fresh keratinocyte suspension (KS) are very similar to those after 22 h 'recovery', and thus presumably to those of the cell surface *in vivo*. This conclusion is similar to that reached using adrenaline stimulation of adenyl cyclase activity as a 'functional' test of membrane receptor integrity (Gommans *et al.*, 1979b). These data also confirm the metabolic viability of our keratinocyte preparations.

Comparison of our absolute values with those reported previously (see, for example, review by Sharon & Lis, 1975) is of particular interest since the present data are apparently the first quantitative measurements pertaining to cells of epithelial origin. It is clear that our figures, at least for ConA and WGA, are an order of magnitude higher than those found for erythrocytes or lymphocytes, corresponding more closely to those reported for cultured fibroblast lines. This may, of course, reflect the unusually large surface area of keratinocytes rather than any specific difference in surface composition. Investigations are currently in progress to establish whether changes in the lectin-binding profile accompany the normal processes of keratinocyte differentiation or occur in pathological situations such as psoriasis.

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Synthesis and turnover of membrane glycoconjugates in monolayer culture of pig and human epidermal cells

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SUMMARY

The regeneration and turnover of the surface glycoconjugates of trypsin-prepared pig and human cultured epidermal cells have been determined using the glycoprotein precursors N-acetyl-D-(1-^{3}H) glucosamine (^{3}H-NAG) and N-(^{3}H)-acetyl-D-mannosamine (^{3}H-NAM). Sialic acid assays have been performed on similar unlabelled cells. The major points which emerged from this study were:

(1) Trypsin-damaged cell surfaces are rapidly repaired, probably by normal membrane turnover. There was a 12% regeneration of sialic acid within 2 h and total resynthesis occurred within 24 h.

(2) The presence of an internal membrane system, part of which also demonstrates turnover, probably contributes to the speed of surface membrane repair. Some of the glycoprotein/sialic acid of this internal membrane system (30%) remains bound for a considerable length of time.

(3) The membrane turnover maintains the cell in equilibrium so that total loss equals the synthesis of glycoprotein.

(4) The equilibration of ^{3}H-NAG or ^{3}H-NAM uptake between 24 and 48 h is limited by the relative concentrations of glucose and labelled sugar in the medium at this time.

(5) ^{3}H-NAM was a more specific marker of glycoprotein than ^{3}H-NAG.

(6) The results for human epidermal cells closely matched those for pig epidermal cells, indicating that pig cells can be used as a model for human cells.

The basal layer of the epidermis has a permanent population of dividing cells whose progeny undergo specific patterns of differentiation (keratinization) on their way up to the surface and are eventually shed. As the epidermal cells migrate upward from the basal layer, they lose their mitotic potential to a large extent and begin to synthesize filamentous and non-filamentous keratin proteins, and thickening of the cell membrane occurs (Montagna & Parakkal, 1974). In view of this, the behaviour of the epidermal cell surface in culture is of particular interest.

Three points have been investigated:

(1) The speed of recovery of the epidermal cell membrane from trypsin treatment.

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(2) The subsequent fate of the membrane glycoconjugates, i.e. turnover.
(3) A comparison of pig and human epidermal cells.

It is indisputable that the conventional trypsin preparation of cells for culture affects their peripheries, but does not cause widespread cytoplasmic damage (Weiss, 1966). The regeneration of the surface membrane and its subsequent metabolism in tissue culture can be studied using labelled precursors of membrane glycoproteins. King & Gray (1978) found that major membrane-bound glycoproteins in pig epidermal cells are labelled with D-(^{3}H) glucosamine hydrochloride. Both N-acetyl mannosamine and N-acetyl glucosamine have been shown to be precursors of the plasma membranes of mammalian cells (Bennet & Leblond, 1977; Warren & Glick, 1968). The surface glycoconjugates of eukaryotic cells are present as glycoproteins, glycolipids and proteoglycans. Since epidermal glycolipids contain mainly glucose (Gray & Yardley, 1975), they are unlikely to be labelled with either glucosamine or mannosamine. Since sialic acid is a common component of membrane glycoproteins (Hughes, 1976), estimations of sialic acid throughout the culture period will give an indication of the recovery of the cell surface. The sialic acid residues of membranes are mainly situated externally and a large amount can be removed by neuraminidase (Hughes, 1976), or by mild acid hydrolysis (Schauer *et al.*, 1975).

MATERIALS AND METHODS

Preparation of epidermal cells

Pigs' ears were thoroughly scrubbed with soap and water prior to shaving with a safety razor. They were then swabbed with 70% ethanol to sterilize the surface. Slices of skin 0·2 mm thick were cut using a Davies dermatome and incubated at 4°C overnight in a solution of 0·25% DIFCO trypsin in phosphate-buffered saline pH 7·2 (PBS) (Oxoid, London SE1). The epidermis was separated from the dermis and shaken vigorously in PBS to free the basal and spinous cells. The suspension was filtered through sterile nylon gauze, hole size 20 μm (J. Staniar & Co., Manchester) and centrifuged at 600 g for 3 min. The cell pellet was resuspended in Eagles Minimum Essential Medium (MEM) containing 20 mM Hepes buffer, 10% fetal calf serum, 0·05 mg/ml gentamicin (all from Flow Laboratories Ltd, Irvine, Scotland) and 5 μg/ml Fungizone (E.R.Squibb & Son Ltd, Wirral, Cheshire).

Human skin was obtained from cosmetic surgery operations and from amputated legs. If the skin biopsies were sufficiently thin, they were incubated in trypsin immediately. Thick pieces of skin tissue were dermatomed as described above.

Radioactive labelling of cultures

The sialic acid precursors N-acetyl-D-(1-^{3}H) glucosamine (specific activity 20 Ci/mmol) or N-(^{3}H)-acetyl-D-mannosamine (specific activity 500 mCi/mmol) (Radiochemical Centre, Amersham, U.K.) were added to the culture medium giving a final concentration of 0·5 μCi/ml. The cell density was adjusted to approximately 2×10^6 cells/ml and 5 ml portions of the labelled suspension were introduced into 25 cm^2 plastic falcon flasks (Sterilin, Teddington, Middlesex). Triplicate cultures were set up for estimation of incorporation of label at time intervals. Parallel unlabelled cultures were set up for protein and sialic acid estimations.

The unlabelled chaser which replaced the original labelled medium after 24 h in one series of cultures, contained an excess (10×) of N-acetyl mannosamine (10 nmol/ml) or N-acetyl glucosamine (0·25 nmol/ml) compared to the labelled medium. The fresh medium which replaced the original growth medium in another series of cultures contained labelled N-acetyl mannosamine at 0·5 μCi/ml (1 nmol/ml) or N-acetyl glucosamine at 0·5 μCi/ml (0·025 nmol/ml).

Harvesting and counting of labelled cultures

Cultures were harvested at intervals up to 72 h and the adherent cells removed by scraping with a silicone rubber 'policeman'. The labelled cells were washed twice with PBS and precipitated with 10% trichloroacetic acid. The pellets were resuspended in PCS scintillating fluid (Hopkin & Williams, Sandbach, Cheshire) and (^{3}H) incorporation measured. Estimation of protein was by Lowry's method (Lowry *et al.*, 1951). A control sample of dead cells was prepared by immersing a sample in boiling water for 1 min and then adding labelled medium. Any non-specific adsorption of the label could then be measured.

Cultures were examined throughout the experiment using a phase contrast microscope and viability assessed morphologically.

Passage of pig epidermal cells

When pig cell cultures had reached confluency (within 48 h), they were subcultured in order to demonstrate that the cells were capable of division. Addition of 0·25% DIFCO trypsin was made to a bottle containing a confluent culture and incubated at 37°C for 15 min. The cells detached and were centrifuged and resuspended in fresh growth medium. They were then seeded into two or three new bottles and incubated at 37°C for 2 or 3 days.

Treatment of pig epidermal cells with neuraminidase

Neuraminidase (Vibrio cholerae, Hoechst Pharmaceuticals, Hounslow, Middlesex) was obtained as a solution of 1 iu in 1 ml sodium acetate buffer (0·05 M, pH 5·5). Mucoprotein (porcine stomach from Sigma Chemical Co. Ltd, Poole, Dorset) was used as a model substrate and pure N-acetyl neuraminic acid (NANA) (Koch Light Laboratories Ltd., Colnbrook, Bucks) as the standard. The optimum conditions for the neuraminidase (NANase) to split the NANA from the cells were then determined. Serial dilutions of NANase, from 0·01 iu to 0·5 iu/ml, were made in phosphate buffer at pH 7 or sodium acetate buffer, pH 5·5. The enzyme was incubated with a constant number of cells at 37°C for 20 min or 40 min. A blank of enzyme and buffer was also set up. Sialic acid assays were then performed on the supernatants.

Labelled epidermal cells were harvested after intervals of 0, 2, 4, 6, 24, 48 and 72 h in culture, centrifuged and washed twice with PBS. Controls of heat-killed cells were also set up as above and harvested at corresponding intervals. They were then resuspended in a solution of 0·1 iu NANase in distilled water and incubated for 20 min at 37°C. The cell residue and incubation medium were separated by centrifugation and both retained for scintillation counting.

Unlabelled cultures were similarly treated and sialic acid assays carried out on the supernatants.

Sialic acid assays

Cultures were harvested at the timed intervals stated above, washed twice, resuspended in 1 ml distilled water and stored at −20°C. For sialic acid estimations, the cells were hydrolysed in 0·1 M HCl (1–2 ml) in sealed tubes at 80°C for 50 min. After cooling 1 M NaOH was added to neutralize and samples adjusted to pH 7·5–8·0. An aliquot was diluted × 10 for protein estimation (Lowry *et al.* 1951). The remaining hydrolysate was centrifuged and the supernatant used for sialic acid assay.

The fluorimetric method of Murayama *et al.* (1976) using pyridoxamine/Zn was chosen for its sensitivity, linearity and relative freedom from interfering compounds, but was scaled down to the range required (0–2 μg) by the use of microcuvettes. An addition of 2 ml reagent* was made to 0·1 ml portions of neutralized hydrolysate, heated in screw-capped tubes for 45 min at 70°C and cooled in ice water. Tubes were then centrifuged for 15 min at 3,000 r.p.m. to remove haze, which if

[*] 2·5 mg pyridoxamine HCl; 25 mg Zn acetate, 1 ml pyridine in 5 ml redistilled methanol.

not removed resulted in poor replication and linearity. Fluorescence was measured on a Vitatron MPS fluorimeter using a 395 nm primary and 470 nm secondary filter.

The concentration of salt (NaCl) derived from acid hydrolysis and subsequent neutralization was critical. A molarity of 0·1 NaCl quenched fluorescence by 50% relative to sialic acid standards in water. The salt molarity of blanks, standards and hydrolysates was as uniform as possible.

In view of initial doubts as to specificity, assays based on thiobarbituric acid were also tested. That of Warren (1959) lacked the required sensitivity and was subject to interferences by compounds other than sialic acid. The fluorimetric modification of Hammond & Papermaster (1976) was highly sensitive but also subject to interference unless the hydrosylates were treated prior to assay with the resin purification process of Schauer *et al.* (1975) (Table 1). The multiple procedures involved, however, led to less consistent results than the simpler pyridoxamine method.

TABLE 1. Sialic acid assays of cultured pig epidermal cells using the fluorimetric thiobarbituric acid method of Hammond & Papermaster (1976) and the resin purification process of Schauer *et al.* (1975)

Culture time (h)	μg Sialic acid/mg protein	
	Untreated	Resin purified
0	7·8	0·8
6	6·0	0·9
24	3·6	2·6
48	6·4	4·7
72	8·1	6·4

Estimation of labelled glycoprotein in the growth medium

To recover any labelled glycoprotein released by the cultured cells, the growth media from 24 h and 48 h cultures were collected. The protein was precipitated with an equal volume of 10% TCA washed twice with distilled water, and the radioactivity measured. A control of labelled growth medium without cells was also set up, to check for binding of labelled sugar to serum components.

RESULTS

Resynthesis of the glycoprotein removed by the trypsin preparation of the cells was evident following 2 h in culture (Figs. 1 and 2). Within 2 h, 30% of the maximum radioactivity (c.p.m./mg protein) of the (^{3}H) mannosamine (^{3}H-NAM) and 20% that of the (^{3}H) glucosamine (^{3}H-NAG) was present in the cells. The radioactivity of the ^{3}H-NAG then increased to reach a peak at 24 h when a plateau was reached. After 72 h the radioactivity had decreased by 17%.

The uptake of ^{3}H-NAM was slightly different. Following the rapid initial incorporation up to 2 h there was a gradual increase, until at 24 h only 65% of the maximum radioactivity was present. The peak was reached 8 h later than that of ^{3}H-NAG. 20% of the added ^{3}H-NAG was incorporated into the cells as compared to 18% of the ^{3}H-NAM at 24 h. The incorporation of both precursors was almost the same at 48 h (6×10^4 c.p.m./mg protein).

After 72 h in culture, the cells became moribund and dropped off the culture surface. This did not

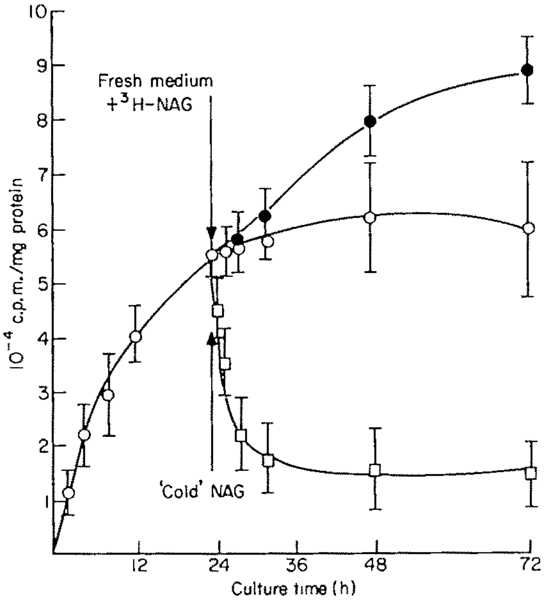

FIGURE 1. Incorporation of N-acetyl-D-(1-^{3}H) glucosamine (^{3}H-NAG) into pig epidermal cells: (○) control; (●) fresh labelled medium added at 24 h; (□) 'cold' chase at 24 h.

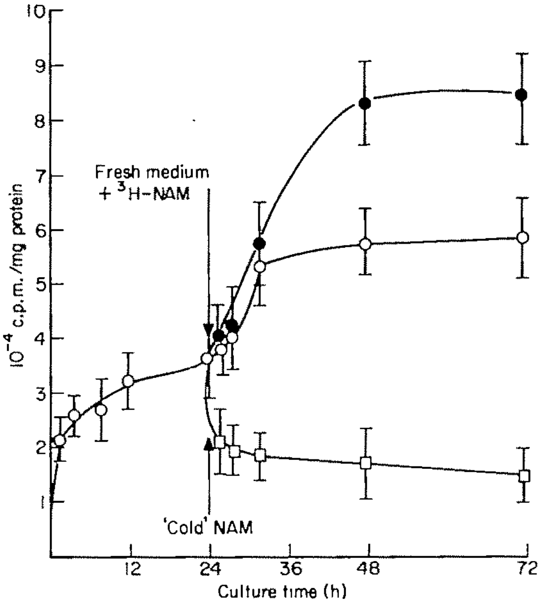

FIGURE 2. Incorporation of N-(^{3}H)-acetyl-D-mannosamine (^{3}H-NAM) into pig epidermal cells: (○) control; (●) fresh labelled medium added at 24 h; (□) 'cold' chase at 24 h.

occur when the growth medium was replaced with fresh labelled medium at 24 h. The cells then incorporated the fresh label to reach a peak 30% higher than that of 'unfed' cultures. The uptake stabilized at 72 h as in 'unfed' cultures.

When unlabelled 'chase' medium replaced the labelled medium at 24 h, the radioactivity of the cells dropped sharply within 6 h and subsequently levelled off to 25% of the control levels at 48 h and 72 h.

The non-specific adsorption of labelled medium by heat-killed cells was 70% lower than that of live cells at 24 h.

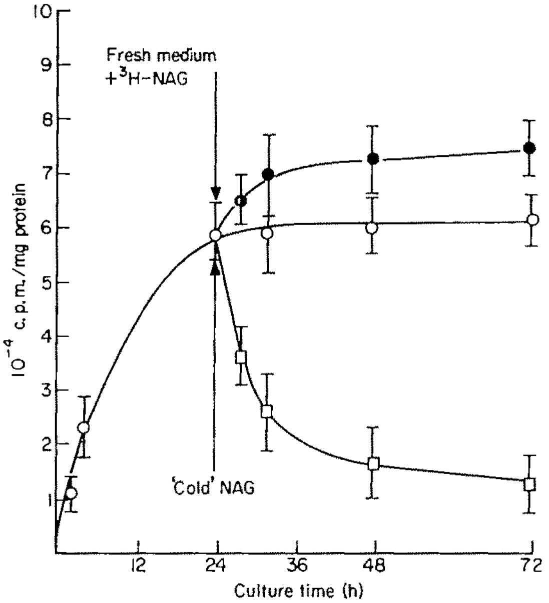

FIGURE 3. Incorporation of N-acetyl-D-(1-^{3}H) glucosamine (^{3}H-NAG) into human epidermal cells: (○) control; (●) fresh labelled medium added at 24 h; (□) 'cold' chase at 24 h.

The uptake of ^{3}H-NAG by human epidermal cells showed a similar pattern to that of pig cells (Fig. 3). The radioactivity reached a peak at 24 h, thereafter levelling off to 72 h. The effect of the unlabelled 'chase' also matched that of pig cells, namely a steep drop in radioactivity within 8 h, followed by a stabilization to 25% that of control cultures.

Sialic acid assays

Following trypsinization, the zero time level of sialic acid was 2·2 μg/mg protein (Fig. 4). Over a period of 24 h, this figure increased to 6·7 μg and subsequently to 6·9 μg/mg protein at 48 h. A series of assays using neuraminidase instead of acid hydrolysis gave similar results. Treatment of 24 h culture cells with neuraminidase removed sialic acid approximately to the zero time level (1·8 μg/mg protein). This was consistent with the assumption that sialic acid was being measured.

Estimation of labelled glycoprotein in the growth medium

The incorporation of labelled glucosamine into the cell glycoproteins and subsequent release into the growth medium was measured in control cultures and those which had been 'chased' 24 h

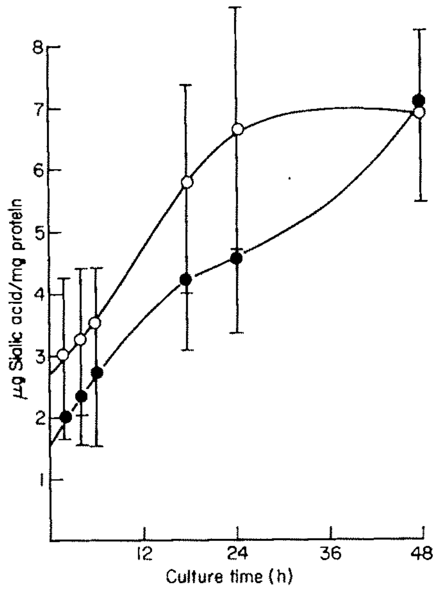

FIGURE 4. Resynthesis of sialic acid by monolayer cultures of pig epidermal cells: (○) sialic acid released by acid hydrolysis; (●) sialic acid released by neuraminidase treatment (0·1 μ/ml for 20 min).

TABLE 2. Estimation of labelled glycoprotein in the growth medium

Culture time (h)	Total counts (c.p.m.)	
	Cells	Growth medium
24	$4{\cdot}5 \times 10^3$	$4{\cdot}7 \times 10^3$
48	$6{\cdot}1 \times 10^3$	$6{\cdot}05 \times 10^3$
48 ('chased')	$2{\cdot}5 \times 10^3$	$3{\cdot}9 \times 10^3$

previously (Table 2). The growth media from both 24 h and 48 h cultures showed similar total counts to the corresponding culture. The 'chased' culture showed a marked decrease in counts (60%) as did the growth medium (40%). The blank of serum and labelled sugar showed very little non-binding of labelled sugar (5% of 48 h culture medium counts).

Treatment of labelled cultures with neuraminidase in order to release membrane bound sialic acid

It was found that incubation of the cells with 0·1 iu neuraminidase (NANase)/ml at 37°C for 20 min released the maximum amount of sialic acid. The pH of the buffer (5·5–7·2) did not affect the result. Following this treatment, the radioactivity remaining in the residues of labelled cultures and also in the released sialic acid was measured (Fig. 5). It can be seen that after 2 h in culture, 20% of the label was bound to the cell residue and not susceptible to NANase action. After 48 h in culture this figure increases to 60% in cells labelled with ^{3}H-NAG and to 35% in ^{3}H-NAM-labelled cells.

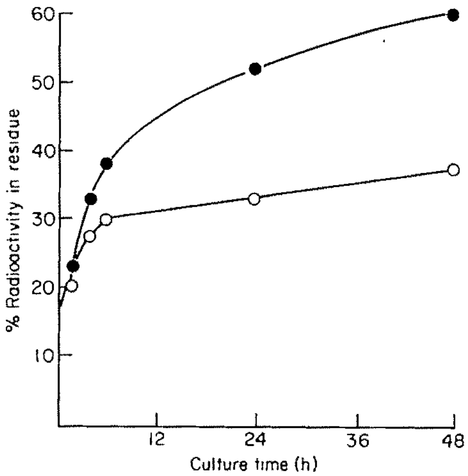

FIGURE 5. Treatment of labelled cultures with neuraminidase (0·1 iu/ml for 20 min) and estimation of radioactivity remaining in cell residue: (●) ^{3}H-NAG; (○) ^{3}H-NAM.

DISCUSSION

Three major points can be emphasized from the results of the present study.

(1) Trypsin-damaged cell surface components are probably repaired by normal turnover of membrane, including an internal membrane system. During the trypsin preparation of the epidermal cells for culture, some components of the cell wall are probably removed by the enzyme (Moscona, 1963). However, the cells rapidly recovered from this treatment and were stuck and spread on the culture surface within 2 h. Weiss (1966) found that trypsin-treated cells began to regenerate factors affecting their contact reactions almost immediately. It follows that there must be synthesis of the glycoproteins removed by trypsin and this is seen in the 20–30% increase in the radioactivity of the cell glycoprotein within 2 h of culture in medium labelled with ^{3}H-NAG or ^{3}H-NAM. This coincided with an increase of 12% in the sialic acid present. Studies on the repair of bulk membrane glycoproteins after enzyme damage (Hughes, 1976) suggested that individual monosaccharides were not replaced *in situ* but that the complete glycoprotein was replaced. This would account for the rapid recovery of the cells from trypsin treatment as would the finding of Doyle *et al.* (1978) of an internal membrane system containing glycoproteins in hepatoma cells. Part of this internal membrane-bound glycoprotein was transferred to the plasma membrane within 3 h while another part remained associated with the internal membrane system for a considerable length of time. In the present study it was found that freshly trypsinized cells contained 32% of the final sialic acid present at 48 h. Therefore, the enzyme does not remove all the cell membrane sialic acid, some of which is probably bound intracellularly (Glick, Comstock & Warren, 1970). Likewise, the cold 'chase' did not totally remove the labelled precursor from cultures, some 25% remaining bound in the cells.

(2) There is a rapid turnover of membrane components in epidermal cell cultures. After 24 h in culture, a subconfluent network of cells is obtained and the repair of the epidermal cell membrane following enzyme treatment is apparent as an increase in the quantity of sialic acid present up to 24 h. Little increase takes place in the 24–48 h period, the cells presumably being in equilibrium. Some cells probably divide during this time since, if such a culture is passaged, the cells can initiate new cultures. When such cultures were 'chased' in a medium containing excess cold precursor, 50% of

the radioactivity of the labelled cells was lost within 24 h. Warren & Glick (1968) also found a fall of 50% in the specific activity of plasma membrane proteins in cells cultured at high density for 24 h. Some of this loss of labelled material might be predicted by a doubling of membrane material with each cell division and hence dilution of label (Scanlin & Glick, 1978). However, the present results show a recovery of 60% of the labelled components in the growth medium of 24 h pre-labelled cells. It is probable that growing and non-growing cells synthesize the same amount of membrane. In the growing cell this remains incorporated into the membrane, perhaps to repair damage, but in the non-growing cell the labelled material is degraded at the same rate at which it is incorporated. Kapeller *et al.* (1973) suggested that surface macromolecules are continually peeling off the normal cell surface during turnover and may be related to growth factors present in conditioned medium.

When fresh labelled medium was added to the sub-confluent cultures at 24 h, the radioactivity of the cells increased. Despite the fact that the cells appear healthy and are capable of division, the growth medium may be depleted of some essential nutrient, leading to a decline in synthetic ability. This nutrient may be glucose, since Eidam and Merchant (1965) have shown that supplementary glucose prolongs the period in which L cells can exist in a healthy state in plateau phase at high cell concentrations. Yurchenko, Ceccarini & Atkinson (1978) also found that the (^{3}H) label equilibration of glycoprotein was increased when the growth medium was renewed. It was suggested that in a closed system such as a culture bottle, the concentration of glucose and monosaccharide label can effect the rate of incorporation of label into macromolecules. In the case of utilization of radioactive glucosamine or mannosamine, the ultimate radioactivity of the glycoproteins formed from the labelled sugar is in part regulated by the relative concentrations of glucose and glucosamine or mannosamine in the medium.

(3) Pig epidermal cells are a valid model for human epidermal cells. The incorporation of labelled glucosamine into both types of cell was very similar, as was the subsequent turnover of membrane components. This fact is of interest due to the unpredictability of the supply of human material. Large quantities of pig skin can usually be obtained which is of particular relevance in biochemical studies.

An additional fact that emerged from the present study was the observation that (^{3}H) N-acetyl mannosamine (^{3}H-NAM) was a more specific membrane marker than (^{3}H) N-acetyl glucosamine (^{3}H-NAG) since the radioactivity of the ^{3}H-NAG in the residue following neuraminidase treatment was 40% higher than that of ^{3}H-NAM. The ^{3}H-NAG must be incorporated into other sugars in the cytoplasm as well as those in the cell surface. Monaco & Robbins (1973) have shown that N-acetylmannosamine is a fairly specific precursor for the sialic acid residues.

The behaviour of epidermal cells from diseased skin, especially those of disordered keratinization, would be of interest using this method. It is possible that the metabolism of the surface glycoprotein may play a role in the pathogenesis of such disorders.

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Molecular basis of substrate and inhibitory specificity of tyrosinase: phenolic compounds

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SUMMARY

In order to investigate the molecular basis of substrates, and the inhibitory specificity of tyrosinase, a large series of phenolic compounds have been analysed by using a High Performance Liquid Chromatographic-Scanning Spectrophotometric system. Depending on their chemical structure, phenolic compounds may act as substrates or as competitive inhibitors of tyrosinase. The ability to act as substrates requires the presence in the molecule of electron donor groups, while competitive inhibition on the contrary requires the presence of powerful electron acceptor groups.

Certain phenolic compounds used as therapeutic agents or as food preservatives are chemically capable of acting as alternative substrates or competitive inhibitors of tyrosinase *in vitro*; their effect on melanocytes *in vivo* therefore merits investigation.

Tyrosinase is an enzyme widely distributed throughout the biological spectrum of eukaryotes; by catalysing the oxidation of phenolic substrates, it leads to the formation of natural biopigments. It is generally accepted that in mammals the pigment formation involves complex reactions in which tyrosinase catalyzes the conversion of the monophenol tyrosine to the diphenol dihydroxyphenylalanine (Dopa), as well as the dehydrogenation of Dopa to the corresponding orthoquinone. The orthoquinone, because of its reactivity, constitutes the key intermediate of the entire process of melanogenesis and undergoes further modifications even in the absence of tyrosinase.

In addition to natural substrates, other phenolic compounds may interact with tyrosinase, as in the case of depigmenting phenolic compounds which, as has been shown by spectrophotometry (Riley, 1969b), are alternative substrates for tyrosinase. Recently, it has also been shown that straight chain C_8 to C_{14} dicarboxylic acids, which *in vivo* are toxic for hyperfunctioning or abnormally proliferating melanocytes, are *in vitro* competitive inhibitors of tyrosinase (Nazzaro-Porro & Passi, 1978; Nazzaro-Porro *et al.* 1979a, 1979b).

In the framework of a systematic study on melanogenesis and its alterations, the effect of mushroom and mammalian tyrosinase on several classes of chemicals has been studied by using analytical methods sensitive enough to allow the isolation and characterization of minute amounts of short-lived intermediates. In this paper we report some results obtained with phenolic compounds and discuss the molecular basis of their specificity as substrates or inhibitors of tyrosinase.

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MATERIALS AND METHODS

Mushroom tyrosinase, grade III, was obtained from Sigma. Mammalian tyrosinase (T^3) from mouse melanoma was 8000 times purified according to the method of Nishioka (1978). Other reagents were obtained from Merck or Sigma. The incubation mixture consisted of 10^{-3} mol/l substrate with 20–40 UI/ml of tyrosinase in 0·02 mol/l K,Na phosphate buffer (pH 6·8) at 25°C. 20–50 μl samples of the mixture were taken at different incubation times and rapidly injected into a reversed phase ODS Hypersil RP-18, 5 μm column of a Hewlett Packard liquid chromatograph.

A scanning spectrophotometer operating from 190 to 540 nm directly connected to the chromatograph allowed spectral characterization of the peaks corresponding to the intermediates. Initial velocities were calculated from the decrease of the area of the peak corresponding to the initial substrate. The half-life of the quinone intermediates was also studied and will be reported elsewhere.

In the present paper we define as 'substrate' a phenolic compound which, under the above conditions, gives origin to a quinone intermediate identifiable on the chromatogram. Non-substrate phenolic compounds were tested as inhibitors. Preliminary studies, employing Dopa as substrate, have shown that all the non-substrates tested were either inactive or behaved as competitive inhibitors for tyrosinase. Further experimental details are given in the table captions.

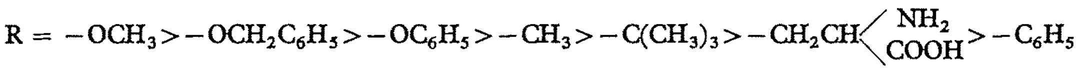
RESULTS

Mushroom tyrosinase

Several substances have been shown to be substrates for mushroom tyrosinase. The values corresponding to the representative compounds are reported in Table 1.

Paramonophenols. It is evident that the basic requirement which allows a paramonophenol to act as substrate for tyrosinase is the presence of an 'electron donor' R group in the paraposition with respect to the OH group. The electron donor property is a function of both the inductive (+I) and the mesomeric (+M) effect of the R group. The greater the electron donor nature, the better the paramonophenols act as substrates for tyrosinase.

We report the following scale of some R groups, arranged in decreasing order according to their electron donor nature:



$$R = -OCH_3 > -OCH_2C_6H_5 > -OC_6H_5 > -CH_3 > -C(CH_3)_3 > -CH_2CH\genfrac{}{}{0pt}{}{NH_2}{COOH} > -C_6H_5$$

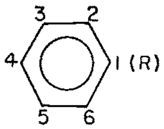
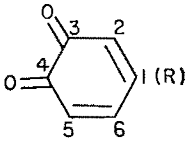
The initial veolocity (measured as described above) for these compounds follows the same sequence.

Halogens have a modest electron acceptor nature, due to the sum of the inductive (-I) and mesomeric (+M) effects. Nevertheless, parachlorphenol and parabromophenol are still oxidized by tyrosinase (Table 1).

When R is represented by an 'electron acceptor' group (−I; −M) the paramonophenol compounds act as competitive inhibitors for tyrosinase. In Table 2 the Ki of some phenolic competitive inhibitors are reported.

If, in the molecule, the position 3 is occupied by a group $\neq$ H, which is not a hydroxyl group, the molecule is not recognized by the active site of tyrosinase. In this connection, antioxidants like 2-terbutyl-4-methoxyphenol (BHA) or 2,6-ditertiarybutylparacresol (BHT) or a pesticide like Joxinil (2,6 diiodo-4-cyanophenol) are neither substrates nor competitive inhibitors of tyrosinase.

TABLE 1. Phenolic substrates for tyrosinase

	 Ring substitution				 Maxima of absorbance (nm)	
Compound	4	3	2	1(R) Electron donor group	Compound	Quinone
Hydroquinone monobenzylether	OH	–	–	$-OCH_2C_6H_5$	287	255–413
4-Hydroxyanisole	OH	–	–	$-OCH_3$	281	255–413
4-Hydroxyphenetole	OH	–	–	$-OC_2H_5$	279	255–412
p.Tertiarybutylphenol	OH	–	–	$-C(CH_3)_3$	275	273–399
p. Cresol	OH	–	–	$-CH_3$	277	256–395
Paracetamol	OH	–	–	$-NHCOCH_3$	240	277–436
p. Chlorophenol	OH	–	–	–CL	280	277–405
Hydroquinone	OH	–	–	–OH	286	246–298 (*p*.benzoquinone)
Catechol	OH	OH	–	–	276	390
1-Tertiarybutyl-catechol	OH	OH	–	$-C(CH_3)_3$	275	268–401
1-Isopropylcatechol	OH	OH	–	$-CH(CH_2)_2$	279	272–399
N-AcetylDopamine	OH	OH	–	$-CH_2CH_2NHCOCH_3$	280	260–394
Pyrogallol	OH	OH	OH	–	267	314
Gallic acid (3,4,5-trihydroxy benzoic acid)	OH	OH	OH(5)	–COOH	260	255–450

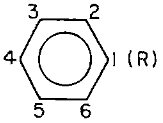
Conditions for quinone intermediates separation: RP-18, 5 μm, ODS Hypersil column (Browlee Labs. Inc.). Column temperature: 40°C. 0·5 ml/min isocratic elution with 30% CH_3CN, 70% phosphate buffer 0·02 mol/l, pH 6·8 for monophenols; 0·5 ml/min isocratic elution with 5% CH_3CN, 95% phosphate buffer 0·02 mol/l, pH 6·8 for polyphenols.

The absorbtion spectra of the quinones were directly obtained by the scanning spectrophotometer connected to the liquid chromatograph at the top of the peak of quinone elution.

Metamonophenols. The activity as substrates for tyrosinase of metamonophenols is much lower than that of the corresponding paramonophenol substrates. They are slightly oxidized by tyrosinase and give rise to the same orthoquinone as the corresponding para-monophenols.

Orthodiphenols. These compounds are particularly interesting because they are both substrates and products of the first reaction when tyrosinase acts on monophenols. That is, the first product of the reaction is also a substrate and is rapidly transformed into a quinone by the enzyme. The sequence of the reaction parallels the oxidation of L-tyrosine by tyrosinase (L-tyrosine→L-Dopa→Dopa-quinone→melanin). An example, according to Riley (1969b), is the case of *p*. hydroxyanisole (see Fig. 1). When L-Dopa acts as substrate for tyrosinase, the rate of the oxidation (formation of Dopachrome) is about seven times more rapid than when L-tyrosine acts as substrate. In the same way, all orthodiphenols are more rapidly oxidized by tyrosinase than the corresponding monophenols. For the representative orthodiphenols tested we report the absorbance maxima of the respective quinones (Table 1).

TABLE 2. Phenolic competitive inhibitors of tyrosinase



Compound	Ki × 10^{-4} mol/l	Ring substitution			
		4	3	2	1 (R) Electron acceptor group
p.Nitrophenol	1·20	OH	–	–	$-NO_2$
p.Hydrobenzoic acid	1·32	OH	–	–	$-COOH$
p.Hydroxybenzaldehyde	1·50	OH	–	–	$-CHO$
Methylparaben (p.hydroxybenzoic acid methylester)	4·08	OH	–	–	$-COOCH_3$
Ethylparaben	4·50	OH	–	–	$-COOC_2H_5$
Propylparaben	4·97	OH	–	–	$-COOC_3H_2$
p.Nitrocatechol	2·83	OH	OH	–	$-NO_2$
3,4-Dihydroxybenzoic acid	2·95	OH	OH	–	$-COOH$

The competitive inhibition was measured using various amounts of L-Dopa in 2·5 ml of 0·1 phosphate buffer at pH 6·8. The concentration of each drug was 2 × 10^{-4} mol/l. The initial rate of linear increase of optical density at 475 nm, due to formation of Dopachrome, was measured at 25°C.

The above considerations concerning the presence of electron donor or acceptor groups are valid also for orthodiphenols. In fact, diphenols like paranitrocatechol and 3,4-dihydroxybenzoic acid, having a strong electron acceptor R group (NO_2 and COOH respectively), act as competitive inhibitors of tyrosinase (Table 2).

In addition, we report in Table 3 a list of orthodiphenols giving rise to red aminochromes (different from dopachrome) and the maxima of absorbance of their respective quinones. A prerequisite for the formation of the aminochrome is the presence in the molecule of nucleophilic amino ($R_1 = H$) or imino ($R_1 \neq H$) groups in α position. These groups are favourably located so that they can attack the 6 position of the orthoquinones, produced by tyrosinase during the first stage of oxidation, giving rise to indole rings (aminochromes).

If $R_3 = H$ (as is the case of Dopamine, 4-hydroxyamphetamine, tyramine, N-methylDopa) the corresponding aminochrome readily gives rise to a black melanic precipitate.

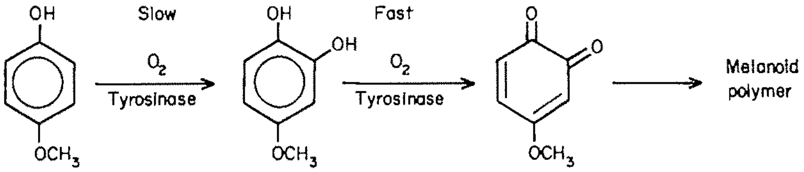
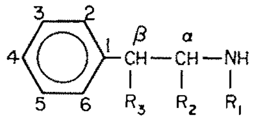
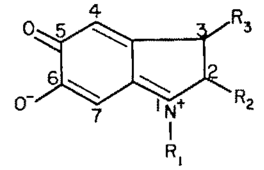


FIGURE 1.

TABLE 3. Phenolic substrates for tyrosinase giving rise to aminochromes





Compound	Ring substitution					Maxima of absorbance (nm)	
	4	3	R_3	R_2	R_1	Compound	Aminochrome
Adrenaline	OH	OH	OH	H	CH_3	279	302–485
Noradrenaline	OH	OH	OH	H	H	278	290–482
Dopamine	OH	OH	H	H	H	280	297–470
Nordefrin	OH	OH	OH	CH_3	H	278	298–478
Isoproterenol	OH	OH	OH	H	$CH(CH_3)_2$	279	300–483
4-Hydroxyamphetamine	OH	–	H	CH_3	H	276	298–482
Tyramine	OH	–	H	H	H	276	297–470
Octopamine	OH	–	OH	H	H	273	293–483
Synephrine (Sympathol)	OH	–	OH	H	CH_3	273	300–500
L-Tyrosine	OH	–	H	COOH	H	275	305–475
L-Dopa	OH	OH	H	COOH	H	280	305–475
DL-3,4-Dihydroxyphenylserine	OH	OH	OH	COOH	H	280	298–486
N-MethylDopa	OH	OH	H	CH_3 αC COOH	H	279	306–483

Conditions for aminochrome intermediate separation were the same as used for polyphenols (see Table 1).

If $R_3 \neq$ H (as is the case of adrenaline, noradrenaline, isoproterenol, etc.) black precipitates are not formed, even after several days. Hence it would appear that an absence of a steric hindrance in the 3 position is indispensable for the formation of eumelanins.

Also, if R_1 is an electron acceptor group, so that the entire imino group is rendered non-nucleophilic (as is the case of N-acetylDopamine), the indole ring cannot be formed.

Paradiphenols. Hydroquinone behaves as a substrate for tyrosinase, the oxidation product being represented by parabenzoquinone. This (a stable quinone) is able to oxidize L-Dopa directly to melanin, in the same way as the oxidation by tyrosinase. A redox reaction takes place, the parabenzoquinone being reduced to hydroquinone. About 3 moles of parabenzoquinone are quantitatively reduced to hydroquinone by 1 mole of L-Dopa, in less than 1 h.

Metadiphenols (Resorcinol). Quinones are aromatic diketones in which the two ketonic groups are linked by a system of conjugated double bonds. A metaquinone structure cannot exist. Consequently, since it cannot form a quinone, resorcinol is not a substrate for tyrosinase. Binding to the active site is excluded by evidence that resorcinol is not a competitive inhibitor of tyrosinase.

Triphenols. Phloroglucynol (1,3,5-trihydroxybenzene) shows a more marked reducing property and tendency to react in solution in the carbonilic tautomeric form than resorcinol. It acts neither as substrate (for the same reasons as resorcinol) nor as competitive inhibitor of tyrosinase, but, when incubated in equimolar amount with L-tyrosine or L-Dopa, in the presence of tyrosinase, it prevents the formation of dopachrome and the precipitation of melanin. Under these conditions, a labile yellow chromophore is formed, which undergoes nucleophilic attack by phloroglucynol, with the formation of new products.

Other triphenols (with different ring substitutions) like pyrogallol (1,2,3-trihydroxybenzene), 1,2,4-trihydroxybenzene, 2,4,4-trihydroxyphenylalanine (6-hydroxyDopa), 2,4,5-trihydroxy-phenethylamine (6-hydroxyDopamine) are easily oxidized by tyrosinase, with the initial formation of the corresponding quinones (orthoquinone for the first, paraquinones for the last three substances).

The 3,4,5-trihydroxybenzoic acid (gallic acid) and its esters (methyl, ethyl, propyl, butyl, octyl, dodecyl gallates) are substrates for tyrosinase (Table 1) in spite of the presence of a strong electron acceptor R group and in this way differ from the mono and diphenols.

Mammalian tyrosinase

Because of the difficulty in obtaining large amounts of purified enzyme, only two compounds were tested with mammalian tyrosinase. Both L-tyrosine and parahydroxyanisole are substrates and give origin respectively to Dopachrome and paramethoxyorthoquinone. With the low levels of enzyme available, the formation of the latter compounds requires several hours and occurs with a long lag period. This sluggishness of the reaction could explain why it has been suggested (Okun *et al.*, 1976) that the initial step of melanogenesis, i.e. the orthohydroxylation of substrates, requires the presence of peroxidase.

CONCLUSIONS

The following conclusions can be drawn from the present study:

(a) The activity of tyrosinase on phenolic compounds depends upon the chemical structure and, in particular, on the electronic properties of the substituents. The capacity of phenolic substances to act as substrates requires the presence of electron donor groups; the capacity to act as competitive inhibitors requires instead the presence of electron acceptor groups.

(b) All the known depigmenting phenolic compounds, which are also melanocytotoxic (Jimbow *et al.*, 1974; Bleehen *et al.*, 1968; Riley, 1969a; Gellin *et al.* 1970; Frenk & Ott, 1971; De Souza *et al.*, 1978) are very good alternative substrates for tyrosinase.

(c) A large number of phenolic compounds, many of which are commonly used as therapeutic agents or as preservatives in food and drug preparations, have a chemical structure which allows them to act as alternative substrates or as competitive inhibitors of tyrosinase *in vitro*. Their effect on melanocytes *in vivo* merits further investigation.

ACKNOWLEDGMENT

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Pharmacology and Treatment

Severe disorders of keratinization: effects of treatment with Tigason (etretinate)

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SUMMARY

Twenty patients with disabling disorders of keratinization were treated with Tigason (etretinate, Ro 10-9359), an oral aromatic retinoid, and the clinical responses and the effects on the skin were monitored. Most patients showed a considerable clinical improvement within 4 weeks. Side effects, such as cheilitis, were common but mostly transient or minor. In the skin there was a decrease in glucose-6-phosphate dehydrogenase activity within the granular cell layer of the epidermis, and an increase in mean epidermal thickness and mean corneocyte area. However, there was little apparent effect on epidermal proliferation or on histological and ultrastructural appearances. These findings suggest that the drug acts at a late stage of epidermal differentiation.

Certain congenital disorders of keratinization are physically and socially disabling. The treatment for these diseases, which include severe ichthyosis vulgaris, lamellar ichthyosis and erythrokeratoderma variabile, has been unsatisfactory and the patients have often been condemned to lead lives of incapacity, discomfort and isolation. Vitamin A has profound effects on epithelial differentiation and the synthesis of more potent and less toxic vitamin A derivatives ('the retinoids') has suggested a new approach to the treatment of these disorders. In this investigation we have studied the clinical response of a group of patients with severe congenital disorders of keratinization to one such drug, Tigason, and monitored its effects on the skin.

PATIENTS AND METHODS

Patients attending the dermatology clinic of the University Hospital of Wales with severe persistent disorders of keratinization characterized by marked hyperkeratosis and/or scaling were considered for entry into the study. If their condition caused considerable disability, had failed to respond to traditional treatments and they (or their parents) gave consent after full explanation and discussion,

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TABLE 1. Clinical details of patients

Diagnosis	Number of patients	Clinical responses at 4 weeks: Excellent	Good	Fair	Poor
Pityriasis rubra pilaris	3		2		1
Autosomal dominant ichthyosis	4		1	2	1
Erythrokeratoderma variabile	3	3			
Darier's disease	3	1		2	
Localized epidermolytic hyperkeratosis	2		1	1	
Lamellar ichthyosis	2		2		
Follicular ichthyosis	2				1
Tylosis	1		1		

they were then included. Twenty patients were finally treated with Tigason between April 1979 and July 1980; details are given in Table 1.

All patients were investigated and treated according to the following standard protocol. The patients were admitted for a 24–48 h period prior to starting treatment, having used only bland local applications in the previous 2 weeks. The investigations outlined in Table 2 were performed. The patients were then given Tigason in a dose of 1 mg/kg body weight per day in two divided doses. The same bland topical applications that they had previously used were permitted if required during the treatment period. Clinical assessments were made initially and at 2 and 4 weeks, and the clinical response at 4 weeks is given in Table 1. At the 4-week assessment, patients were again admitted and the investigations outlined in Table 2 were repeated. At this time it was decided whether or not to

TABLE 2. Investigations performed before and after 4 weeks of treatment

Test	Comment
Full blood counts	Routine
Urea and electrolytes	Routine
Liver function tests	Routine
Skin biopsies from involved and, where appropriate, uninvolved skin for:	
(a) Routine histological examination by light microscopy	Morphological assessment and epidermal measurement using a projection technique (Holt & Marks, 1976)
(b) *In vitro* incubation with tritiated thymidine and subsequent autoradiographic preparation (Shahrad & Marks, 1976)	Epidermal labelling index determination
(c) Frozen for cryostat sectioning and incubating for glucose-6-phosphate dehydrogenase activity (Pearse & Marks, 1978)	Quantitative cytochemical assessment with the Quantimet 720 image analyser
(d) Electron microscopy	Morphological assessment
Measurement of corneocyte areas (Nicholls, King & Marks, 1980)	Mean value obtained from planimetric assessment

continue treatment and at what dose. Patients in whom it was decided to continue the retinoid have been reviewed at monthly intervals for periods of up to 1 year. Biochemical and haematological investigations have been repeated at monthly intervals. In some patients the drug has been temporarily withdrawn to assess if and when relapse occurred.

RESULTS

Clinical responses and side effects

The clinical responses in all the patients are shown in Table 1. Most of the subjects treated began to show improvement after 10 days, and continued to improve at least up to the 4-week time of assessment and in some cases beyond this period (Fig. 1). Patients with autosomal dominant ichthyosis responded more slowly and did not demonstrate maximum improvement before 6–8 weeks. Of the two patients with localized epidermolytic hyperkeratosis, one had diffuse palmoplantar keratoderma and the other linear lesions of the left trunk, arm and leg. In both of these patients and the other patient with tylosis the stratum corneum separated in grossly thickened sheets (Fig. 2). The patients with erythrokeratoderma variabile showed the most dramatic response to treatment. One of these female patients has been remarkably transformed in appearance, and feels that her life has been changed by the drug (Wilkinson, Marks & Finlay, 1980). The results of treatment of patients with this disorder are the subject of a separate report (Finlay & Marks, in preparation). In all patients in whom treatment was temporarily stopped the disorder recurred after 10–20 days. In those patients who have been treated for long periods we have attempted to reduce the dosage and, in the majority of cases, clinical improvement has been maintained at the lower dosage of approximately 0·5 mg/kg/day.

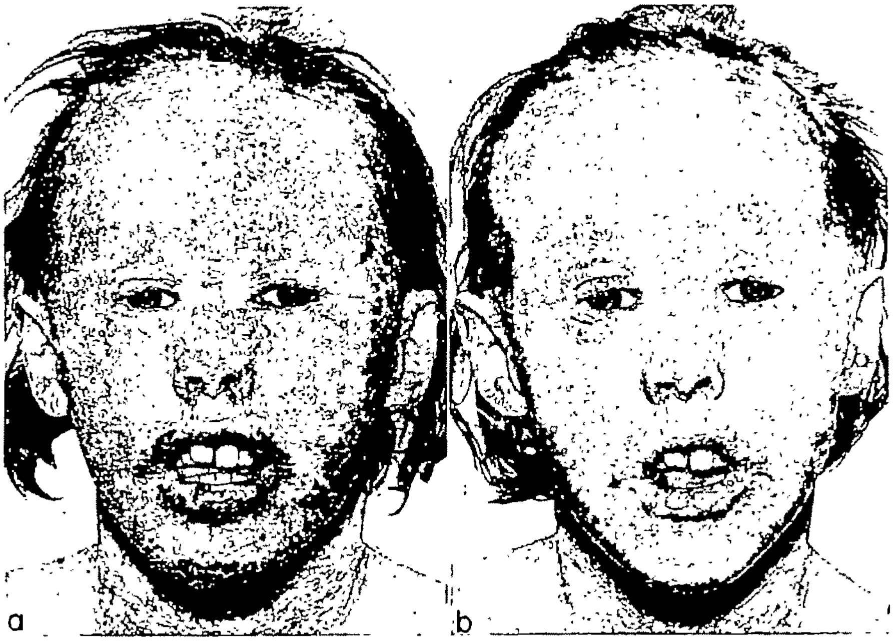

FIGURE 1. Change in appearance of face in patient with lamellar ichthyosis (a) before and (b) after 4 weeks of treatment with Tigason. (Patient DM.)

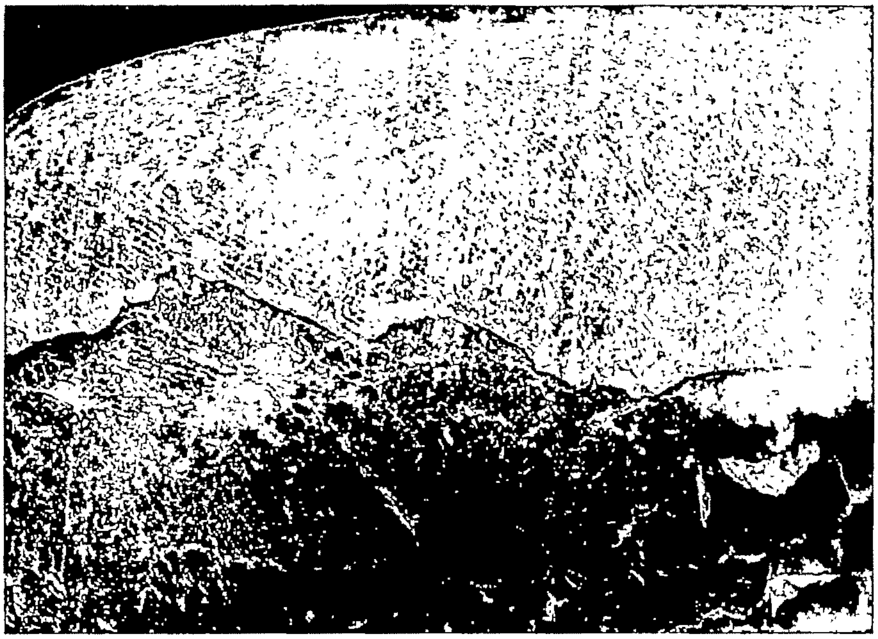

FIGURE 2. Separation of thickened stratum corneum following treatment in patient with localized epidermolytic hyperkeratosis. (Patient CN.)

In general the side-effects were tolerable. The dryness of the lips noted by nearly all the patients did not appear troublesome. The hair loss noted by five patients was not marked and was transient. The nose-bleeds seen in two patients were of a minor nature, stopped spontaneously and did not require treatment. Three patients developed an urticarial or morbilliform rash with the drug. This rash persists while Tigason is taken, clears when the drug is stopped and returns on rechallenge. This is not troublesome and is 'preferred' by the patients to the skin condition for which they are treated. Four patients noticed increased itchiness and in one patient there was transient carotenaemia resulting in a temporary yellow discolouration of the skin. The severity of the side-effects was related to the dosage taken, and in several patients the dosage could be reduced to a level where side-effects were minimal or non-existent, but the beneficial effects of the retinoid were still apparent.

Results of investigations

No haematological or significant biochemical abnormalities were found in any of the patients treated. The histological appearances of the post-treatment biopsies did not differ remarkably as far as the dominant pathological features are concerned, despite the clinical improvement in these patients. However, a thin, patchy granular layer could be discerned after treatment in the biopsies of the patients with dominant ichthyosis in whom it was absent before treatment. The reticular degeneration in the patients with epidermolytic hyperkeratosis and the suprabasilar splitting and dyskeratosis in the patients with Darier's disease persisted in the post-treatment biopsies. The electron microscope appearances of the skin after treatment were similarly unremarkable, although there was more finely granular material between keratinocytes and there was less irregularity in the overall arrangement of the epidermis. The results of epidermal thickness measurments, *in vitro* thymidine autoradiographic

TABLE 3. Epidermal measurements from routine histology before and after 4 weeks treatment with Tigason. The mean epidermal thickness (MET) is recorded as the numbers of epidermal cells in the thickness of the epidermis; the mean keratinocyte height (Mkh) is recorded as the height of the viable epidermal cells in μm

		Pre treatment		Post treatment	
Condition	n	MET (cell no.)	Mkh (μm)	MET (cell no.)	Mkh (μm)
Autosomal dominant ichthyosis	4	4·70±0·47	10·94±0·64	6·03±1·32	10·94±1·44
Pityriasis rubra pilaris	2	8·95	9·84	9·92	9·17
Darier's disease (uninvolved)	3	4·19±0·88	11·97±2·36	5·48±1·89	10·44±1·07
Erythrokeratoderma variabile	2	5·58	9·7	6·68	9·25
All tissues examined	11	5·49±1·97	10·79±1·46	6·70±2·26*	10·17±1·42†

* Significantly different from pre treatment value: 0·01 > *P* > 0·001.

† Not significantly different from pre treatment value.

TABLE 4. Epidermal labelling indices* before and after 4 weeks treatment with Tigason

Condition	n	Pre	Post
Autosomal dominant ichthyosis	4	6·03±2·23	8·58±1·23
Darier's disease (involved)	2	11·22	13·48
Darier's disease (uninvolved skin)	3	6·85±0·59	6·26±2·64
Pityriasis rubra pilaris	2	25·12	20·59
Erythrokeratoderma variabile (involved)	3	14·17±7·26	13·82±3·40
Erythrokeratoderma variabile (uninvolved skin)	2	6.88	7.65
Lamellar ichthyosis	2	17·86	16·09

* Numbers of basal epidermal and suprabasal epidermal cells autoradiographically labelled after exposure to tritiated thymidine.

TABLE 5. Mean corneocyte area before and after 4 weeks of Tigason treatment

Condition	n	Pre (μm^2)	Post (μm^2)
Autosomal dominant ichthyosis	2	1111	1264
Erythrokeratoderma variabile	2	563	919
Pityriasis rubra pilaris	3	733±91	867±271
Lamellar ichthyosis	2	612	638
Localized epidermolytic hyperkeratosis	2	697	948
Darier's disease (uninvolved)	1	971	1139
Tylosis	1	528	646
All tissues examined	13	743±220	917±265*

* Significantly different from pre treatment value: 0·01 > *P* > 0·001.

labelling index determinations and corneocyte area measurements are given in Tables 3–5. To measure glucose-6-phosphate dehydrogenase (G-6-PDH) activity, all samples were reacted in an identical manner and subjected to microdensitometric quantitation on a Quantimet 720 image analyser; activity was reduced after 4 weeks treatment with Tigason. (Pre: $0{\cdot}54 \pm 0{\cdot}08$; Post: $0{\cdot}47 \pm 0{\cdot}13$ optical density units: $0{\cdot}05 > P > 0{\cdot}02$). There were, therefore, significant changes in G-6-PDH activity, epidermal thickness and corneocyte areas in the skin after treatment.

COMMENT

The results of any uncontrolled open study must be viewed with caution. Nonetheless, when the disorders being treated are 'stable' and persistent, the clinical response is as dramatic as in some of the patients in this study and is paralleled by objective alterations in the 'target tissues', the results may still be of considerable importance.

Some of the patients treated in this study have led secluded lives because of the bizarre appearances of their skin and the transformation in some of these has been remarkable and gratifying.

Other reports have appeared which document the clinical responses to Tigason of patients with erythrokeratoderma variabile (Fritsch, 1979), Darier's disease (Burge *et al.*, 1980; Binazzi & Cicilioni, 1979; Orfanos, Kurka & Strunk, 1978; Hönigsmann, Fritsch & Jaschke, 1978) and ichthyotic disorders (Pehamberger, Neumann & Holubar, 1978; Happle, Kastrup & Macher, 1977; Dinet *et al.*, 1978; Fritsch, Hönigsmann & Jaschke, 1978; Viglioglia, 1980). In addition, another synthetic retinoid, 13-cis retinoic acid, has been used to treat patients with this group of diseases (Peck & Yoder, 1976; Gilgor *et al.*, 1980). All the published studies document improvement in the patients treated, beginning some 10 days after starting treatment with relapse occurring after stopping the retinoid. The present report is the first which has attempted to correlate the clinical improvement with the results of detailed studies on the skin.

The full effects and mode of action of retinoids on epithelial tissues have not yet been established, despite much work (Orfanos, 1980). We suggest that the lack of effect on epidermal labelling index measurements or on the histological and ultrastructural appearances of the epidermis in the present study, as well as the clinical response in a wide range of disorders of keratinization, indicates that the drug acts at a late stage of epidermal differentiation. The decrease in G-6-PDH activity within the epidermis and the increase in mean corneocyte area also favour this interpretation.

Side effects are common with this drug but are mostly of a trivial or transient nature. Most patients preferred the minor discomforts of these to their original skin complaint.

It is clear that this retinoid is capable of giving considerable relief to patients disabled by severe disorders of keratinization. Further studies are required to determine the optimal dosage regimen and the detail of the effects of retinoids on the epidermis.

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The efficacy of an aromatic retinoid, Tigason (etretinate), in the treatment of Darier's disease

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SUMMARY

Eighteen patients with Darier's disease were treated for at least 3 months with the oral aromatic retinoid Tigason (etretinate, Ro 10-9359). Seventeen of the 18 patients improved but adverse effects were seen in all patients, and were particularly severe in 6. No haematological or biochemical abnormalities were detected during treatment. We conclude that Tigason is an important advance in the treatment of Darier's disease but adverse effects restrict its use in some patients.

Oral vitamin A (retinol) and topical vitamin A acid (retinoic acid) have been used to treat Darier's disease, but their effectiveness is limited by toxicity (Burgoon *et al.*, 1963; Ashton, Stevenson & Frenk, 1971). More recently, small numbers of patients have been treated with an oral aromatic retinoid analogue of vitamin A (etretinate, Ro 10-9359) with good results (Orfanos, Kurka & Strunk, 1978). We have now studied a larger group of patients and the preliminary results have been published (Burge *et al.*, 1980). Of the forty cases of Darier's disease which have been documented in the Oxford region, a number are mildly affected and do not require treatment, while others have come to terms with their disease and do not want to be treated.

PATIENTS AND METHODS

Eighteen patients, aged 18–77, were treated for a minimum of 3 months. Each patient was assessed by clinical examination; the severity of disease was described as mild, moderate or severe, and photographs were taken. In addition, the patient assessed the severity of his disease on a linear analogue scale. The patients were seen 2, 4, 8 and 12 weeks after starting treatment and examination; linear analogue scale assessment and photographs were repeated on each occasion. Change in disease severity was recorded on a 7-point scale, graded from 0 (no disease) to 6 (extremely severe). Serial punch biopsies were taken where possible (eight patients). The following monthly laboratory investigations were performed; full blood count, ESR, urea and electrolytes, creatinine, liver function tests, blood glucose, cholesterol and urinalysis. After stopping treatment, patients were seen monthly for a further 3 months.

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The results of both the linear analogue and clinical assessment scales were analysed using a non-parametric statistical method.

The initial dose of Tigason varied between 0·5 and 1 mg/kg. When improvement occurred, the dose was reduced. The dose was also reduced, or treatment temporarily stopped, if patients had severe adverse effects.

RESULTS

Darier's disease improved in seventeen out of eighteen patients ($P<0{\cdot}02$). Nine patients were almost completely free of disease after 3 months treatment. However, in six patients the adverse effects were such that, although their Darier's disease improved, their overall condition deteriorated. One withdrew from the study, and in three the improvement was limited by their inability to tolerate the treatment. In one case, the Darier's disease deteriorated during treatment (see Table 1).

Improvement usually occurred within 2 weeks of starting treatment. Acrokeratosis, warty papules and plaques flattened leaving smooth pigmentation, which in some cases disappeared. Three patients noticed that their disease no longer flared up after exposure to sunlight. Both normal and diseased skin became softer in all patients. Nail lesions were not improved and there was little change in palmar pitting.

TABLE 1. Clinical details of patients

	Severity of disease		Change in disease activity score	
Patient	Pre-treatment	After 3 months	(see text)	Adverse effects
1	Severe	Almost clear	+3	Mild
2	Severe	Almost clear	+3	Mild
3	Moderate	Almost clear	+2	Severe
4	Moderate	Almost clear	+2	Severe
5	Severe	Moderate	+1	Severe
6	Mild	Almost clear	+1	Severe
7	Mild	Almost clear	+1	Severe
8	Moderate	Mild	+1	Mild
9	Severe	Almost clear	+3	Mild
10	Severe	Very Severe	−1	Mild
11	Moderate	Almost clear	+2	Mild
12	Mild	Almost clear	+1	Mild
13	Moderate	Almost clear	+2	Mild
14	Mild	Almost clear	+1	Mild
15	Mild	Cleared	+2	Mild
16	Moderate	Almost clear	+2	Mild
17	Moderate	Almost clear	+2	Severe
18	Moderate	Almost clear	+2	Mild

Serial biopsies were taken from eight patients. There was histological improvement after 2 weeks and by 1 month acanthosis, dyskeratosis and hyperkeratosis were almost entirely suppressed. There were still occasional suprabasal clefts and some 'budding' or elongation of the rete ridges in the basal layer. There was often a mixed inflammatory infiltrate in the dermis.

All the patients suffered from adverse effects which were dose related, but in twelve patients these were mild. Six patients were unable to tolerate an initial dose of 1 mg/kg. These patients had severe itching which was accompanied by erosions and excoriations of the softened skin, especially across the shoulders and around the neckline. One patient had an erythematous papular eruption on the previously normal skin of his arms. A biopsy of this showed parakeratosis without evidence of Darier's disease. One of these patients had mild Darier's disease and withdrew from the study. In the others, Tigason was temporarily stopped, or the dose reduced, and two eventually did very well. However the remaining three continued to have problems, although less severe, on low doses.

The commonest adverse effects are shown in Table 2. All the patients suffered from dry lips, while ten patients had a dry mouth or nose. The more severely affected patients had cracking of their lips and nose bleeds. Six patients noticed soreness or itching of the eyes and six patients had some peeling of the palms and soles. Two patients complained that their ears were blocked by excess wax and two patients had friable swollen gums. Increased hair fall was noted in one patient.

TABLE 2. Adverse effects complained of by patients during treatment

Adverse effect	Number of patients
Dry/cracked/sore lips	18
Dry mouth/nose	10
Itching	7
Severe erosions and excoriations	6
Peeling of palms and soles	6
Itching/sore eyes	6
Nose bleeds	4
Drowsiness	4
Thirst	2
Loss of appetite	2
Blocked ears	2
Swollen, friable gums	2
Headaches	1
Hair loss	1
Erythematous papular rash on arms	1

The patient who deteriorated during treatment had particularly severe, malodorous Darier's disease, with florid involvement of the groins, but mild disease of the pre-sternal region. After starting treatment, the skin in this latter area became erythematous, developed coalescent papules, crusted and cracked. Improvement occurred when the dose was reduced, but the skin remained erythematous. Treatment was continued for 3 months with little improvement in his groins. Once treatment was stopped, the skin of his chest rapidly returned to its pre-treatment state.

At the end of the 3 months treatment period, sixteen patients were improved, one had deteriorated and one had withdrawn from the study. Of the fifteen patients who have completed 3 months follow-up, only one has no signs of relapse (Table 3). In six patients there has been some relapse, while in eight the disease has reverted to its pre-treatment severity.

DISCUSSION

The essential abnormality in Darier's disease appears to be a defect in the synthesis, organization or maturation of the tonofilament–desmosome complex, which is associated with fissuring in the epidermis, initially above the basal layer and later extending throughout the Malpighian layer. The

TABLE 3. Numbers of patients relapsing after stopping treatment

	Number of patients relapsed		
Total	1 month	2 months	3 months
15	11	13	14

tonofilaments aggregate around the nuclei instead of aligning themselves in the desmosomes. Fissures are surrounded by acantholytic, dyskeratotic cells (corps ronds) and in the upper layer of the epidermis there are small cells with shrunken cytoplasm (grains).

Vitamin A (retinol) is essential for the maintenance of normal epithelial structure in the skin, upper respiratory and genito-urinary tracts, and cornea. Deficiency causes epidermal hyperkeratinization and hyperplasia, whereas hypervitaminosis is associated with pruritus and desquamation. The mechanism of its action is not fully understood, but it has been shown to stimulate mitosis of epidermal cells, and promote normal keratinization (British Medical Journal 1969; Riley 1969). Although systemic vitamin A has been used to treat disorders characterized by abnormal keratinization (ichthyosis, psoriasis and Darier's disease), it has proved difficult to achieve sufficient local concentration without toxicity (Burgoon *et al.*, 1963). Topical vitamin A acid (retinoic acid) tends to cause irritation of the skin which limits its effectiveness (Ashton *et al.* 1971).

The vitamin A analogues (retinoids) appear to possess greater specificity with less toxicity. *In vitro*, retinoids inhibit proliferation and differentiation of epidermal cells (Madison *et al.*, 1980). They are beneficial in a wide variety of conditions: ichthyosis, erythrokeratoderma variabile, epidermolytic hyperkeratosis, acne vulgaris, psoriasis, pityriasis rubra pilaris and Darier's disease (Orfanos, 1978). It is likely that retinoids act by normalizing terminal epidermal differentiation, rather than by correcting the underlying abnormality. The glucose-6-phosphate dehydrogenase activity within the granular cell layer is reduced after treatment, indicating an alteration in the process of keratinization (Marks, 1980).

Darier's disease is frequently complicated by bacterial infections of the skin and occasionally by Kaposi's varicelliform eruption. It has been demonstrated that patients have depressed cell mediated immunity (Marks, Thor & Lowe, 1978). The principal metabolite (Ro 10-1670) of Tigason is accumulated by lymphocytes and alters lymphocyte function (Bauer & Orfanos, 1980; Soppi, Soppi & Jansen, 1980). Retinoids may exert their effect by reversing the immunological abnormality in patients with Darier's disease, thus reducing secondary inflammation.

There are a number of histological changes in both the epidermis and dermis in patients treated with oral retinoids. The keratinocytes degenerate and desquamate and this is followed by growth of a new population of apparently normal keratinocytes. Langerhans cells have increased numbers of cytoplasmic organelles and are stimulated both in the epidermis and dermis. In addition, there are increased numbers of dermal macrophages and lymphocytes (Schultz-Ehrenburg & Orfanos, 1980; Tsambaos & Orfanos, 1980).

The therapeutic efficacy of Tigason in Darier's disease may be due to effects on both the immunological system and terminal keratinization but the underlying dyskeratosis is probably unaffected.

The margin between a therapeutic and a toxic dose of Tigason is often narrow. A dose of 50 mg per day, which is probably an adequate initial dose for most patients, reduces the incidence of dose-dependent adverse effects. Simple treatment with emollients may also relieve the pruritus and cheilitis, which are the commonest problems. Six patients had particularly severe dermatitis with excoriations and erosions and three of them were sensitive to very low doses of the drug. A papulo-vesicular 'retinoid dermatitis', which is individually dose dependent, is increasingly well recognized (Rufli, 1980).

In one patient, the Darier's disease deteriorated during treatment, but improved when treatment was stopped. He had severe flexural disease but the nail, palm and histological features were those of Darier's disease. Unfortunately the patient declined biopsy during treatment. We noticed a similar deterioration in a patient with Hailey-Hailey disease, who was treated with Tigason, a phenomenon which has been reported by others.

Long-term therapy is likely to be necessary for most patients, but as the mechanism of action and chronic effects of the drug are still unknown, these patients must be carefully monitored. Retinoids are teratogenic in animals, therefore patients should use adequate contraception during treatment and for a year after stopping the drug. Intermittent therapy may be appropriate for some patients.

In conclusion, the oral aromatic retinoids are an important advance in the treatment of Darier's disease, but some patients are unable to tolerate a therapeutic dose because of adverse effects.

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Low-dose PUVA maintenance in psoriasis following Ingram therapy

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SUMMARY

Following the initial treatment of severe psoriasis with conventional Ingram therapy, it is shown that PUVA maintenance at a mean dose-rate of 28·6 J/cm^2/month increases the average period of remission from 7 weeks to more than a year.

Surprisingly, the patients who withdrew from maintenance therapy whilst still in remission have so far continued (10 months) to show an extremely low relapse rate.

PUVA is now accepted as one of the most useful therapies for the treatment of psoriasis, being effective, convenient and relatively free from immediate side-effects. However, certain recent reports of neoplastic changes as possible long-term sequelae following very high doses of PUVA (Bridges & Strauss, 1980; Hofmann, Plewig & Braun-Falco, 1979; Stern *et al.*, 1979) suggest that it is advantageous to use the minimum cumulative dose compatible with therapeutic success.

In this Department, PUVA has been employed for the clearance of established lesions. An alternative approach is now being explored, namely the use of conventional treatment such as Ingram therapy to achieve initial clearance of lesions, followed by minimal-dose PUVA to maintain the remission. Although this investigation is still in progress, certain of our observations seem sufficiently striking to warrant preliminary publication.

MATERIALS AND METHODS

A total of twenty-seven patients have so far participated in this study, which started in January 1978 (Table 1, PUVA-maintenance group). These were selected as being particularly 'difficult' patients, all fulfilling the following criteria: (a) a long history of psoriasis; (b) failure to obtain satisfactory control on an out-patient basis; (c) more than 30% of the body surface involved at the time of examination.

All patients were admitted to the In-Patient Department and were treated according to Ingram (1954) until free from lesions. This treatment consisted of tar-baths, exposure to mercury vapour lamps (UVA + UVB) and the application of dithranol paste, beginning at 0·05% and increasing to a

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TABLE 1. Composition of the PUVA-maintenance and control groups

Group	Number	M/F	Mean age (years)	Mean duration of psoriasis (years)	Classification of psoriasis	
					Vulgaris	Pustulosa
PUVA-maintenance	27	16/11	34·0	16·4	24	3
Control	30	12/18	37·5	10·7	27	3

maximum set by the tolerance of the individual patient. Fluorinated corticosteroids were in general avoided. This phase of treatment usually required about 5 weeks.

Within 3 days of completion of Ingram treatment, PUVA therapy was begun according to the dose-schedules for 8-MOP and UVA described by Wolff *et al.* (1977) and using a Waldmann 6001 cabin (H. Waldmann and Co., Werk für Lichttechnik, Schwenningen). Initially each patient received one treatment per week. In those patients maintaining satisfactory remission for 4–5 months (no more than minimal involvement which appeared to be stable), this was reduced to one treatment on alternate weeks, and after a further 4–5 months to one treatment each 3 weeks. In the case of patients showing any indication of incipient relapse (i.e. the reappearance of significant lesions but less than the criteria defined below) the UVA dose was increased by 0·5 J/cm^2 for skin types I–II or by 1·0 J/cm^2 for skin types III–VI (Wolff *et al.*, 1977), but the frequency of treatment was not changed.

Each patient was examined at intervals of 2–3 weeks. For the purposes of this study, a 'relapse' was defined as the reappearance of more than two palm-sized lesions *or* of multiple nummular lesions of an approximately similar total area; in these cases PUVA was discontinued immediately. Of the patients remaining in remission, maintenance therapy was eventually stopped at the discretion of the investigator or at the request of the patient. As far as possible (fourteen patients), follow-up of this latter group was continued after cessation of maintenance therapy. The average duration of PUVA maintenance (whole group) was 8·7±5·8 months and the mean cumulative dose 249±167 J/cm^2. This corresponded to an average dose-rate of 28·6 J/cm^2/month.

The control group (Table 1, controls) consisted of patients who satisfied the criteria described for the PUVA-maintenance group, but had been admitted to the In-Patient Department for Ingram therapy in the period immediately prior to the present investigation. The period between termination of therapy and 'relapse' (as defined above) for the control group was established by retrospective analysis.

RESULTS

The relapse-rate for the control group is shown in Fig. 1. It is seen that clinical lesions had recurred in 50% of these patients within 7 weeks after completion of Ingram therapy. By contrast, in the PUVA-maintenance group (Fig. 2), only three relapses occurred in total; 75% of the patients were still free from significant lesions one year after discharge from the In-Patient Department. It may be relevant that two of the three relapses were pustular psoriasis.

A remarkable and wholly unexpected finding has resulted from the follow-up of the fourteen patients who stopped PUVA maintenance whilst still in remission and who remained available for regular control. Instead of reverting to the high relapse-rate characteristic of the control patients, the 'post-maintenance' group continues to follow the curve of Fig. 2. So far 12/13 (92%) have remained

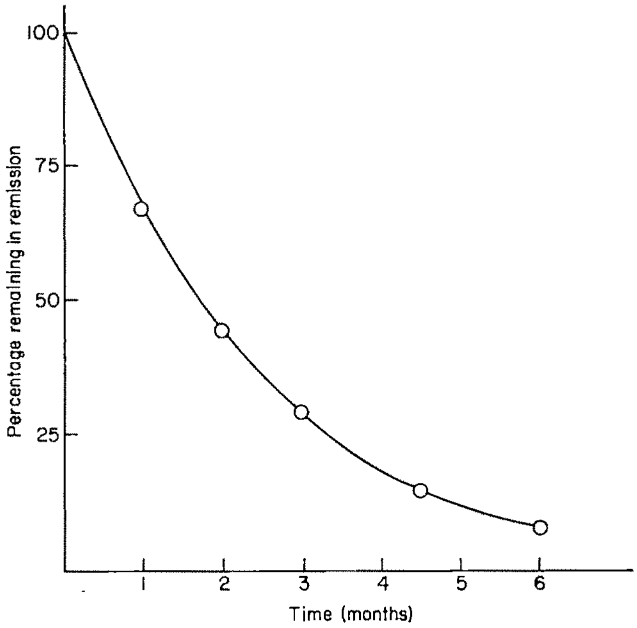

FIGURE 1. Control group (n = 30). Percentage of patients remaining in remission at various times after completion of Ingram therapy.

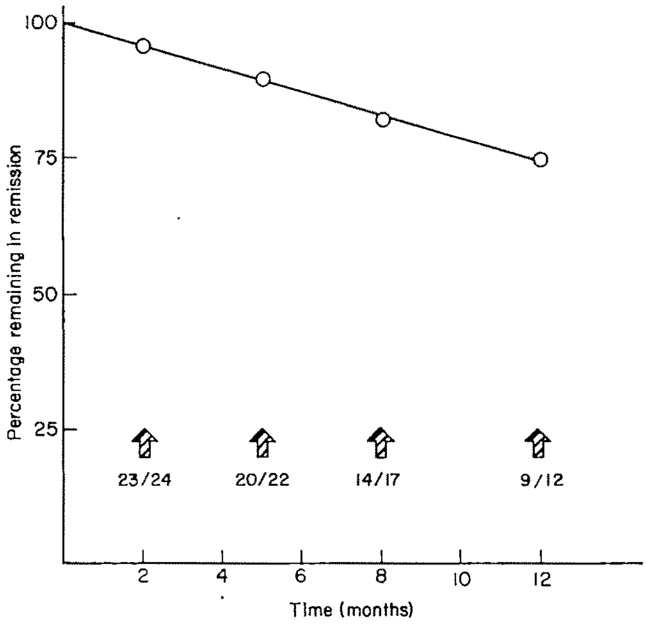

FIGURE 2. PUVA-maintenance groups (n = 27). Denominators of the inset fractions indicate the number of patients remaining in therapy plus the cumulative relapses.

free of lesions 4 months after withdrawal from maintenance therapy, and 7/9 (78%) who have completed 10 months without treatment are still in remission. These figures both differ from the control group at the level $P<0{\cdot}01$.

DISCUSSION

Our findings for the control group are comparable to those of other workers who have studied patients with especially severe psoriasis. For example Vella Briffa *et al.* (1980a) reported that, following Ingram therapy, 74% of such patients relapsed to one-half of their pre-treatment area after 5·4 months. It is seen from Fig. 1 that 74% of our patients had begun to relapse after an average of 3·2 months; it is reasonable to assume that this relapse would reach the stage described by these authors some 2 months later.

The value of PUVA maintenance has already been established following initial clearance with this regime (Wolff *et al.*, 1977; Vella Briffa *et al.*, 1980b). The present investigation demonstrates that PUVA is equally effective for the maintenance of patients cleared with Ingram therapy, an approach which obviously necessitates far lower cumulative doses of UVA.

The observation that 'post-maintenance' patients show a very low relapse rate is surprising. Wolff *et al.* (1977) remarked that certain patients 'remained free of lesions for months despite the fact that maintenance therapy was discontinued', but gave no figures to support this statement, and no further attention has apparently been paid to this aspect of PUVA treatment. Following initial clearance with PUVA (but no subsequent maintenance), the relapse rate is identical to that after Ingram therapy only (Vella Briffa *et al.*, 1980a). It is, of course, possible that the relapse-curve following withdrawal from PUVA maintenance will prove to be sigmoid rather than exponential, but in any case a significant therapeutic advantage seems to be feasible.

It is difficult to postulate a satisfactory explanation at the cellular level without recourse to unlikely hypotheses such as the existence of a discrete sub-population of 'psoriatic' keratinocytes. Nevertheless, these findings strengthen the hope that psoriasis therapy may eventually be directed at modifying the long-term course of the disease rather than achieving a brief remission of symptoms.

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Effect of topical 17α propyltestosterone on sebum excretion in man

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SUMMARY

17α Propyltestosterone produced a mean inhibition of sebum excretion of 20% when it was applied topically for four weeks to the forehead skin of patients with acne.

Increased sebum production is the main pathological correlate of acne (Cunliffe & Shuster, 1969a; Burton & Shuster, 1971) and its inhibition is therefore a major therapeutic objective. As the sebaceous gland may be inhibited by drugs which act on lipogenesis or hormone release or action, the likelihood of serious unwanted effects is high. For this reason a locally acting topical agent would be desirable. 17α Propyltestosterone suppresses the hamster flank organ when applied topically (Ferrari *et al.*, 1978); we have therefore examined its effect on sebum excretion in patients with acne.

PATIENTS AND METHODS

Sebum excretion rate (SER) was measured as previously described (Cunliffe & Shuster, 1969b) in twelve male and thirteen female patients with acne (aged 17–31 years), before and after 4 weeks treatment with 17α propyltestosterone applied twice daily to the forehead as a 4% solution in 70% ethyl alcohol. In fifteen patients the application and measurement were made only in the centre of the forehead and in the remaining ten the application was made on the whole forehead and SER was measured on both sides. All patients continued with their previous medications, none of which included agents known to affect sebaceous gland function. No attempt was made to quantify clinical response and the overall clinical impression only was recorded.

RESULTS

17α Propyltestosterone produced a small but significant reduction in sebum excretion (Table 1) with a mean decrease of 20±6%. There was considerable variation in individual response, but this was not related to the degree of seborrhoea before treatment. There was no obvious change in the clinical appearance of the acne.

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TABLE 1. Sebum excretion rate ($\mu g/cm^2/min$) before and after 4 weeks treatment with 17α propyltestosterone topically twice daily

	Before	After	% Change
Mean ± s.e.	1·62 ± 0·15	1·20 ± 0·12	20 ± 6
Significance (paired t-test)	$P < 0{\cdot}005$		

DISCUSSION

Although androgens are a major stimulus to the human sebaceous gland (Shuster & Thody, 1974; Pochi & Strauss, 1974; Burton, Laschet & Shuster, 1973), topical antiandrogens have for the most part been ineffective as inhibitors of sebum production (Cunliffe, Shuster & Cassells-Smith, 1969; Pye, Burton & Harris, 1976; Simpson *et al.*, 1979) for reasons which remain unclear. However, a decrease of sebum production of 14% was found in male subjects treated with topical methylnortestosterone for 4 months (Strauss *et al.*, 1969). This is similar to the decrease in SER which we found with topical 17α propyltestosterone and it is much less than that produced by this preparation applied to the hamster flank organ (Ferrari *et al.*, 1978). This effect is too little to be useful therapeutically and it might therefore be worth pursuing analogues of 17α propyltestosterone further.

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Case Reports

Sclerosing lymphangitis of the penis: a lymphangiofibrosis thrombotica occlusiva*

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SUMMARY

Three patients with so-called non-venereal sclerosing lymphangitis of the penis are presented. Light and electron microscopy of one case revealed a lymphatic collecting vessel with a fibrin thrombus in the process of recanalization and vessel wall fibrosis due to hyperplasia of smooth muscle cells and fibroblasts. The term 'lymphangiofibrosis thrombotica occlusiva' is proposed. Lymph stasis is suggested as a provoking factor for the dilatation and clinically striking firm thickening of the affected collecting vessel. No microorganisms were recognized. Within the fibrin thrombus, sprouts of endothelial cells showed intracellular vacuoles, probably indicating the first identifiable step in lymph capillary lumen formation. Signs of collagen remodelling were encountered in the thickened vessel wall.

The disorder to be described mostly presents acutely as a painless tender cord- or worm-like lesion in the coronary sulcus of the penis but may also affect the prepuce and the distal part of the penile shaft. The aetiology is unknown and neither the anatomical target nor the underlying pathological process have been fully clarified. The lack of comprehensive and conclusive data concerning pathogenesis is reflected by the various names applied to the disease (Hutchins, Dunlop & Rodin, 1977). Non-venereal sclerosing lymphangitis of the penis, however, is the most commonly used term.

An ultrastructural examination in one of three cases presented here may help to elucidate these problems. The results of electron microscopy in this disorder have not been previously reported.

CASE REPORTS

Case 1

A 26-year-old patient presented with a somewhat worm-like firm lesion of 5 days duration involving

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one-third of the circumference of the inner root of the prepuce (Fig. 1). There was no history of preceding or concomitant penile infectious disease or trauma. A total excision of the lesion was performed. *Light microscopy*: three dilated vessels with intraluminal homogeneous eosinophilic material are conspicuous. One vessel displays a thickened fibrous vessel wall (Fig. 2), the others demonstrate partial thrombi which have been recanalized.

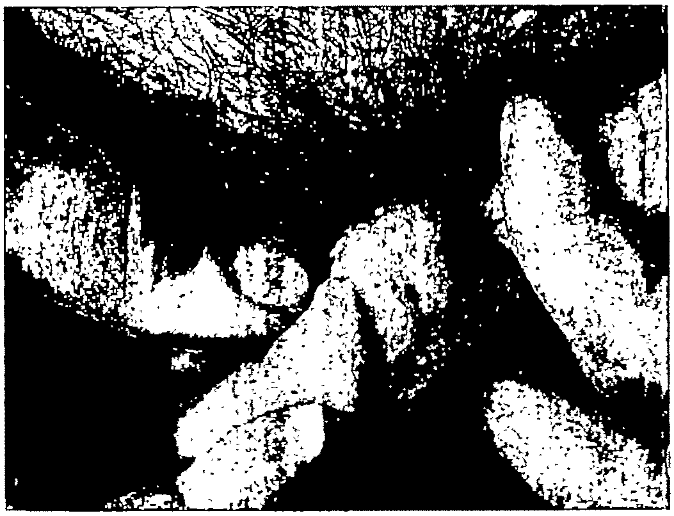

FIGURE 1. Case 1: 26-year-old patient with worm-like firm lesion of 5 days duration.

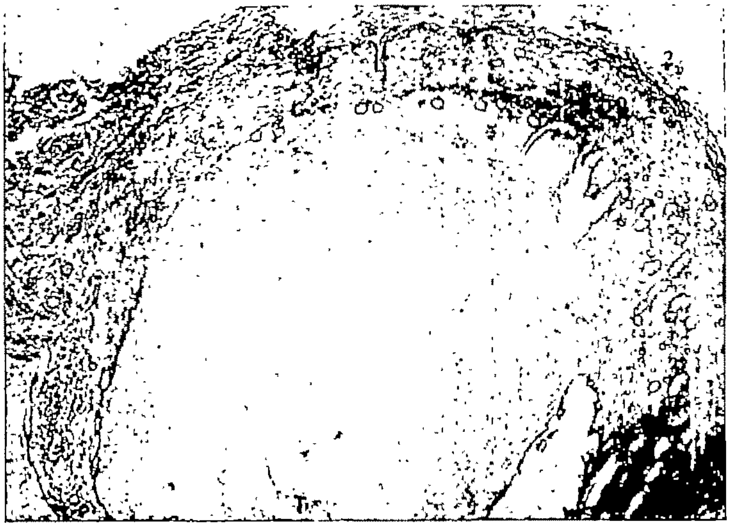

FIGURE 2. Case 1: biopsy demonstrating a dilated vessel with thickened fibrous wall and intraluminal eosinophilic condensed material (H & E, × 13).

Case 2

A 30-year-old patient presented with a prominent firm nodule of two weeks duration just proximal to the coronary sulcus. The history shed no light on the possible cause of the disorder. Histological examination of the biopsy failed to show any vascular changes.

Case 3

A 33-year-old man presented with a worm-like tender lesion on the left side of the distal penile shaft, just behind the coronary sulcus. The lesion had developed 2 weeks previously. There had been no signs of inflammation prior to the onset. Additional details could not be obtained. The lesion was totally excised. *Light microscopy*: examination revealed a vessel with a wide lumen filled with a condensed eosinophilic material. The vessel wall had a thickness of about 0·75 mm and consisted almost totally of collagen fibres, smooth muscle cells, fibroblasts and scattered capillaries filled with erythrocytes. Elastic lamellae were absent as revealed by Elastica-van Gieson stain.

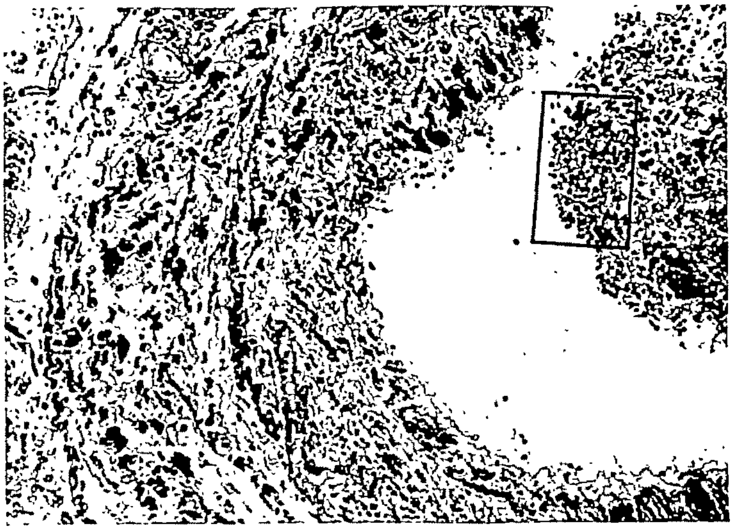

FIGURE 3. Fibrin thrombus in the lumen of a dilated lymphatic collecting vessel with wall fibrosis (semi-thin section, Araldite-embedding, Toluidene-blue, ×32).

Semi-thin sections of Araldite-embedded material displayed a fibrin thrombus occluding the greater part of the lumen (Fig. 3). In between the fibrin network, mononuclear cells and capillaries with lymphocytes in their lumen were easily discernible (Fig. 4). *Electron microscopy* (routine methods)—luminal features: the lumen of the vessel displayed a more or less dense fibrin exudate which was infiltrated predominantly by macrophages; lymphocytes occurred to a lesser degree. Fibrin was often found in invaginations of the cytoplasmic membranes of macrophages, but never seen intracellularly. Capillaries within the fibrin thrombus were lymphatic in nature, showing a flat endothelial cell layer without underlying basal lamina and a wide irregularly shaped lumen displaying flocculent protein material representing lymph and abundant lymphocytes (Fig. 5). Beneath the endothelial cells only fibrin was detectable (Fig. 6). Interendothelial junctions were mainly of gap junction type; however, in places tight and patent junctions were also observed. In some areas oval shaped cells within the fibrin thrombus demonstrated large intracellular vacuoles with thinning of the peripheral cytoplasm

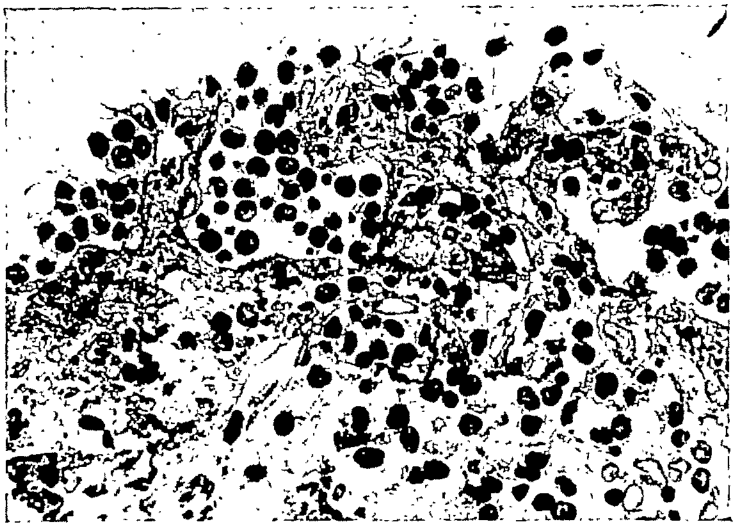

FIGURE 4. Detail of Fig. 5: fibrin thrombus in recanalization; in between the scattered fibrin exudate and mononuclear cells (lymphocytes, macrophages) a lymphatic capillary with endothelial cell lining and many lymphocytes in its lumen (× 128).

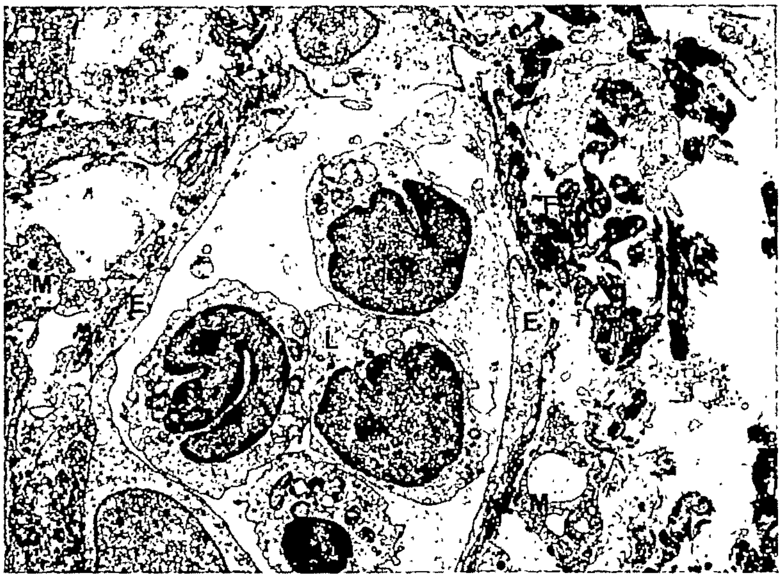

FIGURE 5. Lymphatic capillary within the fibrin thrombus: flat endothelial cell layer (E) without underlying basal lamina. In the lumen lymphocytes (L); outside the capillary are fibrin exudate (F) and macrophages (M) (× 3456).

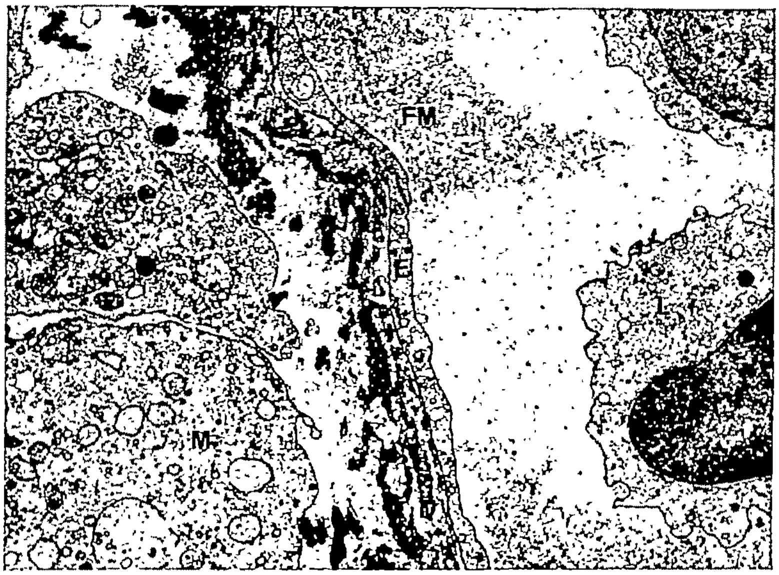

FIGURE 6. Lymphatic capillary demonstrating a flat endothelial cell layer (E). In the lumen lymphocytes (L) and a flocculent material (FM). A basal lamina of endothelial cells is lacking, only fibrin exudate is encountered. Macrophages (M). (× 8000).

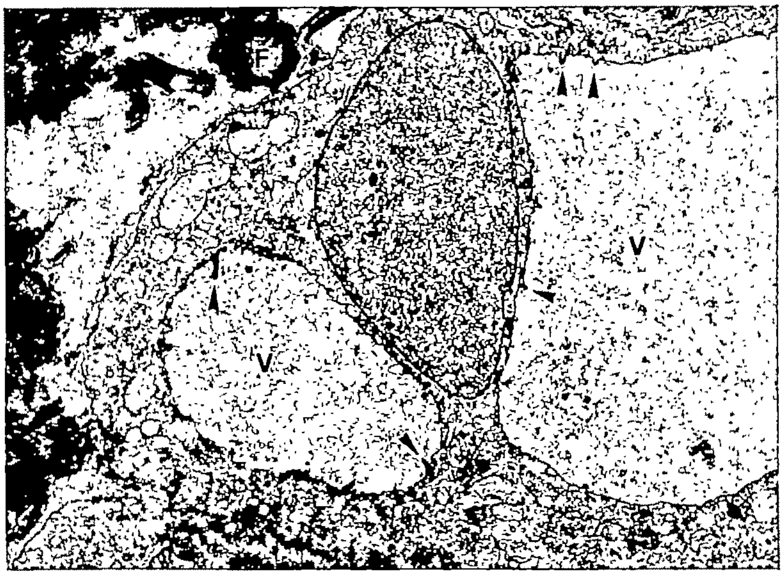

FIGURE 7. Oval shaped cell within the fibrin thrombus (F) showing large intracellular vacuoles (V) lined by a cytoplasmic membrane displaying pinocytotic vesicles and short microvilli-like protrusions (arrows) (× 9128).

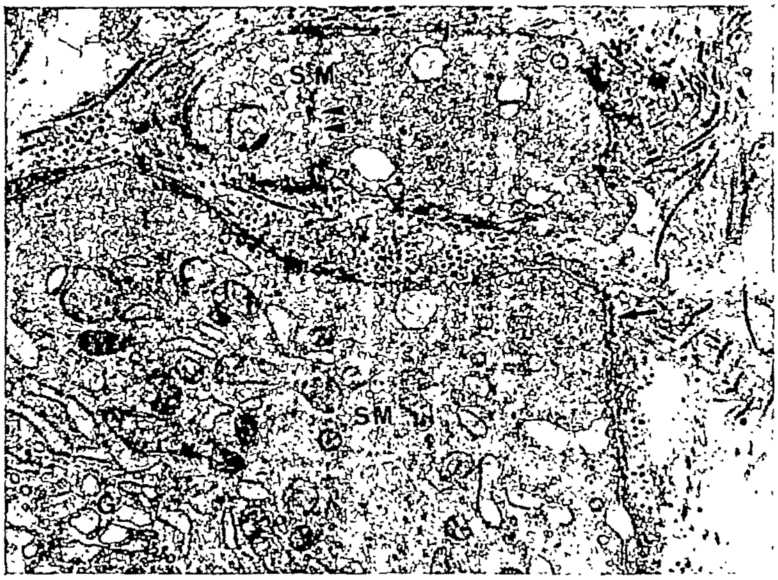

FIGURE 8. Vessel wall thickening from proliferation of smooth muscle cells (SM) and newly formed collagen. Smooth muscle cells partly in close contact (top), showing myofilaments, Golgi apparatus (G), sarcolemmal vesicles (arrowheads) and basal lamina (arrow). (× 17,100).

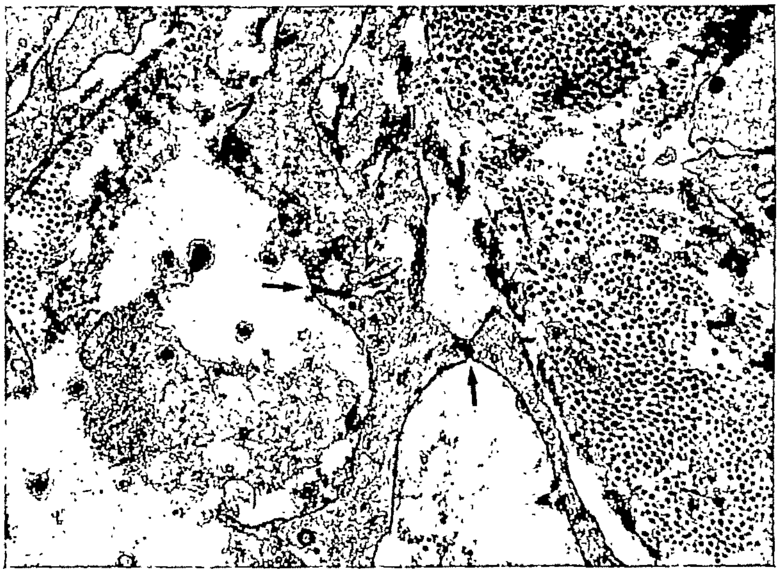

FIGURE 9. Fibroblast-like cells in the vessel wall with gap junction-like structures in between slender cell processes (arrows) (× 16,530).

(Fig. 7). The vacuoles were lined by a cytoplasmic membrane displaying pinocytotic vesicles and short microvilli-like protrusions. The outer cytoplasmic membrane exhibited pinocytotic vesicles to a lesser degree; microvilli and a basal lamina were absent. Vessel wall features: the wall of the affected vessel demonstrated an incomplete endothelial cell lining with a basal lamina mostly in evidence. There were no elastic lamellae separating a subendothelial intimal layer from a tunica media. Closely subjacent to the endothelial layer there was a narrow collagenous zone devoid of cells. Throughout most of the vessel wall there was a dense proliferation of smooth muscle cells and fibroblasts with extensive newly-formed collagen fibrils in between (Fig. 8). Intracellular gap junctions equivalent to type I junctions of Ebner & Gebhart (1972) could be seen between slender extensions of fibroblast-like cells (Fig. 9). Rarely, collagen fibrils were found in membrane-bound vesicles in some of these cells. Blood capillaries possessed endothelial cells with basal lamina; their lumina were filled with erythrocytes and a large number of thrombocytes.

No microorganisms were detected.

DISCUSSION

This localized process affects a lymphatic collecting vessel and not a venous one. This view is supported by the following ultrastructural features:

(1) In the lumen of the vessel, only fibrin, macrophages and lymphocytes are seen and erythrocytes are absent. Proliferating lymphatic capillaries may penetrate only into the media of a blood vessel, the intima layer remaining free (Cliff, 1976; Johnson, 1969). Lymph contains fibrinogen and other coagulation factors and has the ability to form a coagulation thrombus (Courtice, 1972; Földi, 1969; Huth, 1972) which was already known to Rudolf Virchow in 1858.

(2) The vessel wall is markedly thickened due to a hyperplasia of smooth muscle cells and the production of collagen and ground substance by fibroblasts. Lymphatic collecting vessels are morphologically characterized by smooth muscle cell layers closely beneath the endothelial cells (Casley-Smith, 1969; Leak, 1972; Weiss, 1977) which may occupy a basal lamina. A proliferation of smooth muscle cells is a well-known feature in inflammatory or thrombotic lymphatic vessel processes (Altdorfer & Clodius, 1976; Pfleger *et al.*, 1967).

The pathological process is evidently a fibrin thrombus formation with subsequent organization and recanalization. The dilatation and wall thickening of the lymphatic collecting vessel can be considered a secondary phenomenon due to lymph stasis by occlusion. These features are similar to what Pfleger *et al.* (1968) have designated 'thrombolymphangitis productiva' in their histological study on peripheral lymph vessel pathology. Likewise, Kandil & Al-Kashlan (1970) in their case (reviewed by Pfleger) demonstrated histological changes fulfilling the criteria of 'thrombolymphangitis productiva'.

Analogous histological features in the lumen and wall of lymphatics have recently been revealed by light and electron microscopy in experimentally induced lymph stasis (Altdorfer & Clodius, 1976; Magari & Asano, 1978). These equivalent results suggest that lymph stasis could be the provoking factor of the dilatation and clinically striking firm thickening of the wall of the lymphatic vessel. However, which comes first—a thrombosis causing lymph stasis by occlusion or a functional lymph stasis leading to a secondary thrombosis—remains to be elucidated.

The vessel wall fibrosis can be compared with phlebosclerosis developing in the intima layer of venous vessels after prolonged increased hydrostatic pressure. However, as the tunica intima in lymphatic collecting vessels is represented only by an endothelial cell layer, the process may only establish itself in the tunica media. We propose the term 'lymphangiofibrosis thrombotica occlusiva of the penis', illustrating the probable pathogenesis.

The underlying aetiology (microbiological agent?, traumatic insult?) is still obscure. Possibly different aetiological factors evoke a uniform clinical and morphological picture.

The disease runs a self-limiting course. Sparse intracellular collagen fibrils in membrane-bound vacuoles of mural cells with long slender processes may indicate a phagocytic phenomenon. Phagocytosis and degradation of collagen fibrils by fibroblasts and smooth muscle cells obviously play a role in remodelling and turnover of collagenous tissue (Levine, Reddick & Triche, 1978; Staubesand, 1977; Ten Cate & Deporter, 1975; Zeitz, Ruiz-Torres & Merker, 1978). Thus, our findings may indicate an initial degradation of collagen which may finally lead to clinical regression of the vessel wall fibrosis after lymph stasis has vanished (recanalization of the thrombus, collateralization).

The curious phenomenon of large intracellular vacuoles in endothelial sprouts within the fibrin thrombus may indicate the first identifiable step in lymph capillary lumen formation. Analogous features have been recognized in angiosarcomas by electron microscopy (Rosai *et al.*, 1976). This type of primary intracellular lumen formation is fundamentally different from that found in embryonal angiogenesis and in granulation tissue. In both, the vessel lumen forms extracellularly in between endothelial cells.

The occurrence of lymphocytes in lymphatic capillaries of the fibrin thrombus should be emphasized. Lymphocytes constitute more than 80% of the cells in human peripheral lymphatics under normal conditions (Sokolowski, Jakobsen & Johannessen, 1978). These cells have migrated into the peripheral non-lymphoid tissue from the blood vascular system (Hall & Morris, 1963; Morris, 1972; Smith, McIntosh & Morris, 1970). The number of cells in peripheral lymph can be considerably increased by physical activity (Engeset, Sokolowski & Olszewski, 1977) and by inflammation of the drained tissue.

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Allergic contact dermatitis due to an epoxy acrylate

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SUMMARY

A printer developed allergic contact dermatitis due to epoxy acrylate resin. Incomplete cross-reactivity between four epoxy acrylate resins and a lack of cross-reactivity to epoxy resin was demonstrated. The epoxy acrylate resin has been shown to be a potent cutaneous sensitizer for the guinea-pig.

In recent years a new technology has developed in the field of coatings and printing inks known as radiation curing (Shahidi, Trevellas & Vona, 1974; Rybny, 1974; Shahidi & Powanda, 1975). These materials dry after application to a surface by instantaneous curing induced by intense continuous ultraviolet (UVL) emission between the wavelengths 300 and 400 nm.

The system is based on three components. The first is a synthetic resin, such as a polyester, epoxy, alkyd or vegetable oil, which has been acrylated (e.g., an epoxy acrylate (EA)). A reactive monomer acts as a cross-linker which will bind together the reactive high molecular weight (MW) acrylated resins. The most commonly usd monomers are multifunctional acrylic monomers (MFAs) pentaerythritol triacrylate (PETA), trimethylpropane triacrylate (TMPTA) and hexanediol diacrylate (HDODA). The final component is a chromophore such as an aromatic ketone, benzoin or a chlorinated aromatic compound, which absorbs photons of UVL and transfers the energy to the MFA inducing cross-linking and curing of the material. The chemistry of this process has been reviewed recently in this journal (Nethercott, 1978).

REPORT OF THE CASE

A 58-year old printing pressman began operating an experimental press fitted with ultraviolet drying devices. Two months after operating the device regularly, he developed an itchy eruption on the dorsa of his hands, forearms and eyelids. The eruption recurred each time he returned to work.

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CLINICAL INVESTIGATION

Materials and methods

Patch tests were carried out using the chemicals outlined in Table 1. The materials were applied to 'Al-test Strips' (Hollister-Stier Ltd) which were then affixed to the upper back of the subject with Dermicel tape (Johnson & Johnson Ltd). The EA resins had been tested on a panel of eight other human volunteers and were found not to cause cutaneous irritation in the concentrations used.

The patch tests were removed at 48 h. The sites were scored after the pressure effect of 'Al-test Strip' had resolved. The scoring system is outlined in Table 1. A further examination of the sites were carried out at 96 h.

TABLE 1. Patch test reactions at 48 and 96 hours

	48 h	96 h
PETA 0·1% in petrolatum	0	0
TMPTA 0·1% in petrolatum	0	0
HDODA 0·1% in petrolatum	0	0
Methylmethacrylate 1% in petrolatum	0	0
Linseed Oil acrylate 1% in petrolatum	0	0
EA (a) 1% in petrolatum	0	0
EA (b) 1% in petrolatum	2+	2+
EA (c) 1% in petrolatum	2+	2+
EA 5 1% in petrolatum	2+	3+
Epoxy resin (Hollister-Stier Ltd) 1% in petrolatum	0	0

0 = negative; 1+ = macular erythema; 2+ = oedematous or vesicular reaction; 3+ = spreading, bullous or ulcerative.

Results

Table 1 outlines the patch test reactions at 48 and 96 h. No evidence of reaction to MFAs was noted. Reactions occurred to three of the four EA resins but not to epoxy resin. A 2 mm punch biopsy was taken from the site of reaction to EA (Fig. 1).

The biopsy revealed an acute eczematous reaction with spongiosis and a marked dermal lymphocytic infiltrate in keeping with allergic contact dermatitis (Lever & Schaumberg-Lever, 1975).

EXPERIMENTAL INVESTIGATION

Materials and methods

Animals. Albino Hartley-Dalkin strain guinea-pigs weighing 300–500 g were obtained from the Connaught Laboratories, Toronto, Canada. They were housed in metal cages and fed Purina Guinea-Pig Chow and fresh water 'ad libitum'.

Chemicals. The chemicals assessed are listed in Table 2. Commercial grade samples of each were provided by various manufacturers.

Irritation. Ten control animals were exposed to each chemical by intradermal injection in 0·1 ml of propylene glycol. The animals were examined 24 h after injection to ascertain the maximum concentration which did not cause severe cutaneous injury or systemic intoxication.

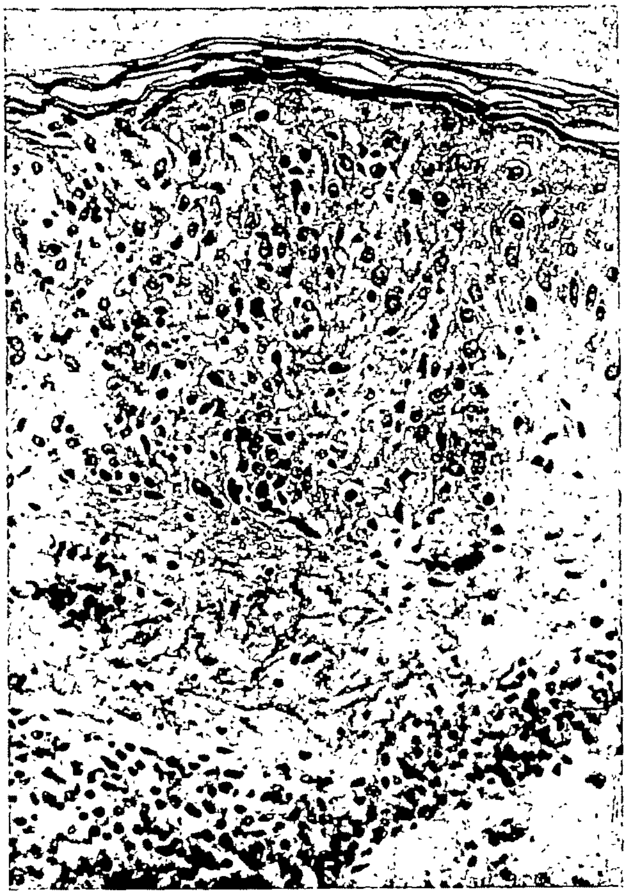

FIGURE 1. Skin biopsy of the positive patch test to EA (b) resin. Note the lymphocytic perivascular infiltrate in the dermis with exocytosis of the lymphocytes into the epidermis producing spongiosis and intraepidermal vesicle formation (× 250).

A further ten animals were exposed to the chemicals at various concentrations in petrolatum. Usually, three concentrations of the chemical being assessed were applied to 2 cm × 2 cm 3 MM Whatman filter paper squares which were then applied to the animals' flanks. These were covered by occlusive tape (Blendern, 3 M Co.), then a 3 inch elastic adhesive bandage (Elastoplast, Smith and Nephew Ltd) was wrapped firmly about the animals' torsos. The bandages were removed after 24 h. At 48 h, the sites were examined for evidence of cutaneous reaction. A non-irritant concentration was ascertained for each chemical which was used later to assess other animals for evidence of sensitization.

Sensitization. The method described by Magnusson and Kligman was utilized (Magnusson & Kligman, 1970).

Each group of animals was sensitized to one chemical only. Induction was carried out in two stages. The first involved intradermal injections into the shoulder regions. A row of three injections of the following composition were made on each side of the animal:

TABLE 2. Experimental parameters and results of the experimental investigation

Chemical	Intradermal dose (mg)	Topical concentration (Gm %)	No. of animals	Challenge concentration	Sensitized (%)	Sensitization grade*	Intradermal sensitizing dose for 50% of the (s.d. 50) mg %	Sensitization potential relative to PETA at s.d. 50
EA1	3	50	10	10	70	5	<3	<0·05
	9	50	10	10	90			
EA2	3	50	10	30	70	5	<3	<0·05
	9	50	10	30	90			
EA3	3	100	10	100	50	5	3	<0·05
	45	100	10	100	60			
	105	100	10	100	90			
EA4	105	100	10	100	40	3	>105	>0·001
EA5	3	100	10	100	0	1	>3	—
EA (b)	3	100	10	100	70	5	>3	<0·05
	15	100	10	100	70			
	30	100	10	100	100			
PETA	0·75	25	15	10	53	5	<0·15	1
	1·5	25	10	10	100			

* Magnusson and Kligman, 1970.

(1) 0·1 ml of adjuvant (Freund's complete adjuvant, Difco Laboratories, Detroit, Michigan).
(2) 0·1 ml of the chemical in propylene glycol in the concentration noted in Table 2.
(3) 0·05 ml of the chemical as in (2), combined with 0·05 ml of adjuvant.

One week later, the shoulder was shaved and the chemicals were applied to the site. The material was applied in petrolatum in the concentration noted in Table 2. It was applied on a 2 cm × 4 cm 4 MM Whatman filter paper which was applied over the sites of the intradermal injections. This was held in place by a layer of impervious plastic tape (Blenderm. 3 M Co.) covered by a 3 inch elastic adhesive bandage (Elastoplast, Smith and Nephew Ltd) wrapped firmly about the animal. All bandages remained in place for 48 h and then were removed.

Challenge. All animals were challenged 2 weeks after topical exposure. A 5 cm × 5 cm of the flank was clipped and then shaved with care to avoid abrasion of the skin. The maximum non-irritant topical concentration established in the control animals was used to challenge the animals. Two experimental groups were also challenged with epoxy resin 1% in petrolatum (Hollister Stier Ltd). The patches were applied and assessed as described above for the assessment of irritancy.

Results

Table 2 outlines the results with seven chemicals. EA1 to EA(b) are all epoxy acrylate resins. Animals that were exposed to EA1 and EA2 were challenged with epoxy resin as well as EAs. No reaction to the epoxy resin was noted in these two groups of animals.

COMMENT

This patient developed eczematous dermatitis of his hands, forearms and eyelids. Similar clinical findings have been previously described with allergy to amines used to cure epoxy resins (Dernehl, 1955, Dernehl, 1963), epoxy resin (Savitt, 1955; Morris, 1957; Grandjean, 1957; Gaul, 1962; Gaul, 1967) and MFAs (Emmett & Kominsky, 1977). This investigation has demonstrated that the individual was sensitized to the EA component of the printing ink. Allergy to MFA was not demonstrated as in two other printers exposed to ultraviolet inks previously reported (Beurey, Monzeolle & Weber, 1976; Nethercott, 1978).

Table 1 illustrates that he did not exhibit hypersensitivity to one of the four EA resins tested. A technical representative of the ink manufacturer stated that EA (a) had a different chemical structure from the other three EAs characterized by more side chains on the polymer. This difference may explain the failure of cross-reactivity. It is possible that a common reactive diluent such as a glycidyl ether, could have been present in the three EA resins that gave positive epicutaneous tests (Fregert & Rorsman, 1964). The manufacturer stated that none of these contaminants was present in any of the EA resins tested. Patch tests with glycidyl ethers were thus not carried out because of the risk of inducing sensitization (Fregert & Rorsman, 1964). Unreacted acrylic acid may have been present in small amounts in the EA resins. If this were the sensitizer, cross reactivity with the MFAs tested should have occurred as they also contain small amounts of acrylic acid. The lack of such cross reactivity suggests that acrylic acid was not the cause of the sensitivity.

No sensitivity to epoxy resin in the patient or the experimental animals was evident. The patch test concentration used in the animal tests should have been satisfactory to demonstrate sensitivity to epoxy resin (Thorgeirson, Fregert & Ramnas, 1978). Such a reaction could have implied that a common low molecular weight of epoxy oligomer was the sensitizer as has been shown for epoxy resins (Fregert & Thorgeirson, 1977). Sensitivity to EA resin seems to be related to the entire molecule and not to the epoxy component or contaminants.

Possibly, EA (a) is a less potent sensitizer than the other three EAs akin to epoxy resins of certain molecular weights which exhibited low sensitization potential (Fregert & Thorgeirson, 1977; Thorgeirson, Fregert & Ramnas, 1978). This awaits verification based on animal investigation and human experience.

When sensitivity to an EA is suspected, testing should be carried out with the specific resin to which the patient has been exposed. Testing with methylmethacrylate or epoxy resin found in standard patch test trays, or with other EA resins than that to which the subject is exposed, may not detect sensitization.

The animal investigation confirms that EAs possess the potential to induce cutaneous allergy (Table 2). PETA has been used in a similar fashion to these chemicals in industry. It is a proven cause of human contact sensitivity (Nethercott, 1978). Comparison of the EAs to PETA should thus be helpful in assessing their potential to induce problems in usage situations.

Using the standard grading system (Magnusson & Kligman, 1970), one is led to the conclusion that all but EA4 and EA5 are similar to PETA. This analysis failed to take dose into consideration.

If one is comparing all the chemicals to PETA, comparison on the basis of the intradermal dose alone reduces the difference in sensitization potential as all chemicals were applied to the animals at more than 25 g per cent which was used in the PETA experiments, while the other experiments utilized higher topical concentrations. Bearing this bias in mind, the differences in dose which will sensitize 50% of the animals, the SD 50s, are striking. The sensitization potentials of the EAs are low compared to PETA. The data were analysed taking topical dose and intradermal dose into consideration, utilizing a PDP 1100 computer with a UNIX user operating system. A model where the proportion of animals sensitized was assumed to be binomially distributed with a constant probability of the animals being sensitized, best fits the data (Baker & Nelder, 1978).

The EAs differ significantly from PETA ($P<1\%$), they are not significantly different from one another except EA5 which exhibited no sensitization potential. Thus the standard scoring system fails to differentiate these strong sensitizers from one another.

Many cases of contact sensitivity to PETA have been reported (Emmett & Kominsky, 1977; Nethercott, 1978). This is the second report of sensitization of EA. This usage experience suggests that the differences demonstrated have practical significance. Consideration should be given to the use of quantitative dose-sensitization relationships when assessing sensitizers using the guinea-pig maximization test (GMT) as Magnusson and Kligman have asserted (Magnusson & Kligman, 1970).

The experimental results with EA5 are of interest. This material was so irritant when given intradermally, that only 3 mg could be administered by this route. It is a less potent sensitizer than EA3 and EA4, but its irritancy precludes giving an adequate dose to investigate fully the material's sensitization potential. As the patient presented here was allergic to EA5, it may be a cutaneous allergen (i.e., the reaction to EA5 could be a cross reaction to sensitivity to one of the other EAs to which the patient had been exposed in spite of the negative GMT). This illustrates why a negative GMT suggests that a chemical has a low but not absent sensitization potential.

Hazard may be defined as the risk of sustaining an injury in a practical usage situation. It would appear that under the conditions in which EA resins are used in the printing and coating industry, the hazard of acquiring allergic contact dermatitis is small. This is in contrast to experience with epoxy resin where one investigation has demonstrated a 35% incidence of sensitization in two hundred and thirty-six workers exposed (Rudzki & Krajewska, 1973). The difference is not in the sensitization potential as both classes of chemicals are potent sensitizers (Fregert & Rorsman, 1964). The amount of skin contact is probably the differentiating factor as skin exposure in the printing trade is much less than in the varied uses of epoxy resin.

This man was able to return to work with the offending inks. He avoided skin contact with the materials, wore protective clothing, washed his skin when it was contaminated and had another worker clean the press 'blankets' between press runs. This favourable experience with a sensitized individual suggests that with attention to proper cutaneous hygiene as outlined in the past for epoxy resins (Morgan, 1957; Key, 1969; Broughton, 1965), the development of cutaneous allergy to EAs should be preventable.

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Cold urticaria with persistent weals

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SUMMARY

A patient with cold urticaria is described in whom weals appeared immediately after an ice cube test for 3 min and persisted for a week as a red, tender swelling. The duration of the oedema was dependent on the intensity of the immediate reaction. If the immediate wealing was blocked by treatment with an oral antihistamine 3 h before the ice cube test, no delayed reaction was seen. Antihistamines given after the immediate wealing had occurred did not influence the delayed reaction. Reactions to intradermally injected histamine, prostaglandin E, kallikrein, serotonin and serum appeared normal.

In patients with cold urticaria the weals provoked by an ice-cube test usually disappear within an hour. We here report studies on a patient in whom the weals persisted for 4–6 days.

CASE HISTORY

The patient is a 47-year-old woman with four children. Her mother and father have diabetes, as have three of eight brothers and sisters. There is no family history of cold hypersensitivity. As a child she had urticaria from eggs and liquorice. At age 7–8, she had urticaria and itching on areas exposed to cold. The weals remained for 20–30 min. After 2 years the hypersensitivity to cold disappeared spontaneously. Some years later, for some time, she had rhinitis when exposed to cats or cows. She had a cholecystectomy when 20 years old. At age 28 she had a severe depression and was treated with insulin. After a time the injections were stopped due to a hypersensitivity reaction with itching and a shock-like picture. She had an appendectomy in 1965 and at that time diabetes mellitus was detected. It was treated with a diet until 1976 when 5 mg of glibenclamide (Daonil) was added.

In February 1976 she developed itchy swellings of the skin when exposed to cold and rain. An ice cube test gave an urticarial reaction which persisted for a week. An antihistamine (clemastine, Tavegyl) stopped the itching but had no definite effect on her urticaria. In December 1979 her sensitivity to cold seemed to be worse. When out in the cold the exposed areas also showed a deeper swelling and when her feet got cold the swelling spread to the groins. She was therefore refered to our wards for investigation.

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Laboratory investigations

A complete blood count was normal as were serum levels of zinc and copper: ESR 9 mm/h; blood sugar 10–14 mmol/1; cryoglobulins were not found but weak cold agglutinins at 4°C; serological tests for syphilis were negative. Radioallergosorbent test (RAST) was positive to insulin but negative to horse, cat, cow, pig, various pollens, milk and egg; IgE 60; C_3, C_4 and C′1 esterase inhibitor were normal; C1q and C1s were increased (136 and 173% of normal); urine sugar 4–100 mmol/l.

Skin tests

An ice-cube test for 30 s showed reddening but no urticaria. After 45 s application a few weals appeared after some minutes. The 1 min ice-cube test produced an even weal which remained as a dark red swelling for 2 days. When the ice-cube was applied for 3 min the swelling remained for a week. It was tender to pressure and slightly itching. Test with a tube with water at 15°C for 3 min induced urticaria after 4 min which remained for 2 days. The same test performed at 37°C and 44°C was negative. Tests for dermographism and pressure urticaria were negative.

Intradermal injection (0·05 ml) of kallikrein 4 iu (Padutin), prostaglandin E (5 μg/ml), serotonin (0·1 mg/ml) and histamine HCl (0·1 mg/ml) showed normal immediate reactions at 15 min which disappeared within 1 h. No delayed reactions were seen the following day. Injection of the patient's own serum (0·1 ml) gave an immediate weal but no delayed reactions.

The effect of oral antihistamines 3 h before the ice-cube test was also investigated. Only one substance was tried per day. As a control an ice-cube test was done before taking the drug. After 4 mg of cyproheptadine (Periaction) some reddening was seen after a 1 min ice-cube test, but no immediate or delayed urticaria. After 1 mg of clemastine fumarate (Tavegyl) there were some weals a few minutes after 45 and 60 s of ice-cube testing. They remained for 1–2 h. After 3+3 mg of dexchlorpheniramine maleate (Polarimin prolong) small weals were seen after an ice-cube for 45 and 60 s. They remained for 1 and 2 h respectively as after clemastine. Ketotifen 1 mg orally showed the same results as clemastine and dexchlorpheniramine. When cyproheptadine or ketotifen was given 3 and 20 h after the ice-cube test no effect was seen on the duration of the weals.

Histology

A biopsy from a 72 h old weal shows normal epidermis and a slight increase of mainly round cells around vessels. There is no real oedema and no signs of vasculitis. The number of mast cells seems normal.

Treatment and course of disease

The various antihistamines tested have offered some help but in winter-time she must stay inside all day and be transported in a warm car to her work. In the summer she has stopped taking antihistamines but she then easily gets urticaria on chilly, rainy days. This summer when passing an open freezer in a shop she developed severe swelling of the face and had difficulties in breathing for 4 h. Such symptoms are rare in winter-time.

DISCUSSION

The patient has an unusual type of cold urticaria which I have not previously seen described. Sarkany & Turk (1965) reported a patient who reacted with erythema and tender indurated areas 24 h after an ice-cube test. The histopathological picture showed perivascular lymphocytes as in a tuberculin type hypersensitivity. Five similar cases were later described by Bäck & Larsen (1978) but the histology showed only a slight increase of mononuclear cells around the vessels. A patient with delayed erythematous deep swelling occurring at 9–18 h after challenge with ice-water for 3 min has also been studied by Soter *et al.* (1977). Here a family study suggested an autosomal dominant mode of inheritance. In none of these cases was there an immediate urticarial reaction as in our case.

Patients with chronic urticaria react to histamine and bradykinin with an immediate weal reaction which sometimes is followed by a delayed swelling and erythema having its maximum after 8–12 h and persisting up to 24–48 h (Juhlin & Michaëlsson, 1969). These patients also reacted with tender oedema and redness 8–24 h after injection of kallikrein. We assumed that histamine caused leakage of plasma into extravascular tissues which activated kallikrein to which the patients showed an increased sensitivity. Such a mechanism seems, however, not to have been involved in the patient described here since she did not show any abnormal reaction to either injected histamine, prostaglandin E, serotonin, kallikrein or serum. After a pronounced cold-induced immediate wealing her reaction is also more prolonged than we have seen in chronic urticaria.

The duration of the reaction seemed to be correlated to the intensity of the immediate weal where an ice-cube test for 1 min gives oedema for 2 days, and a test for 3 min, a reaction for 6–7 days. The immediate reaction could be suppressed by antihistamines. In such cases no delayed reactions were seen. With some suppression of the immediate wealing the delayed oedema was of shorter duration. It was, however, not possible to shorten the duration of the delayed oedema by starting to give antihistamines 3 h after the immediate reaction had occurred. This indicates that other mediators than histamine are involved in the delayed reaction.

From the patient's history it is interesting to note that as a child she had an ordinary type of cold urticaria which spontaneously disappeared and had also had symptoms of acute urticaria to egg and rhinitis to animal dander. Such atopic symptoms are rather common in patients with cold urticaria. She also has a persistent IgE mediated hypersensitivity to insulin which is a problem in controlling her diabetes.

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UV-induced pyrimidine dimers and rosacea

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In a previous paper of ours (Nunzi *et al.*, 1980), we reported the *in vivo* deposition of IgG and IgM at the basal zone and on the collagen fibres of the papillary dermis of the clinically involved skin of rosacea patients. In addition, antibodies eluted from circulating lymphoid cells (CLC) of rosacea patients were found directed to nuclei of epithelial, dermal and endothelial cells, both in involved and non-involved rosacea skin. ANA directed to the same rosacea substrates was also eluted from CLC of normal controls whilst ANA eluted from rosacea patients did not show any activity to normal substrates.

These findings strongly suggested that changes occur in the skin of rosacea patients affecting both collagen fibres and DNA, possibly due to sunlight exposure.

Therefore, the possible occurrence in rosacea skin of UV-induced pyrimidine dimers which could generate ANA was investigated in four sex and age-matched subjects, two of whom had papulo-pustular rosacea and two with apparently normal looking skin.

Detection of pyrimidine dimers utilized the indirect immunofluorescence method. Firstly skin sections were incubated with UV-DNA antiserum (E.11.4; 3) obtained from rabbits as described by Cornelis *et al.* (1977). Secondly the same sections were incubated with FITC-labelled goat anti-rabbit antiserum (anti-IgG_1, -IgG_2, -IgA, -IgM, -IFc and -Fab; Nordic Labs, Tilburg, The Netherlands).

All immunological findings we reported in the previous paper were confirmed, but neither in rosacea nor in control skin were pyrimidine dimers detected.

It is to be noted, however, that the pyrimidine dimers we used to induce our antiserum were obtained through the irradiation with predominantly 254 nm UV. This wavelength is not present in sunlight and it may be that dimers are antigenically different from those supposedly occurring in rosacea.

In fact, inflammation induced by UV-C has been proven to be different from that induced by UV-B, the latter being more persistent (Eaglstein, Sakai & Mizuno, 1979).

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Comment

MIXED CONNECTIVE TISSUE DISEASE

Multi-system disorders of unknown aetiology are often difficult to categorize, particularly those included within the group of 'Connective Tissue Disorders'. Although the diagnosis of well-established rheumatoid arthritis, systemic lupus erythematosus and systemic sclerosis is readily made, there remain patients who disobligingly refuse to conform to any one of our conventional categories but instead possess features corresponding to two or even three connective tissue diseases (Dubois, 1974; White, 1959; Tuffanelli & Winkelmann, 1961). To these the term 'overlap syndrome' has been given, and it is often a matter for controversy whether to regard these syndromes as a variant of a recognised connective tissue disease, or a new co-existing separate disease. Strictly a diagnosis of 'overlap syndrome' should only be applied to patients who independently satisfy current criteria for two distinct connective tissue diseases. An example would be dermatomyositis developing in a patient with well substantiated SLE. Unfortunately 'overlap syndrome' is often loosely applied to patients with multi-organ involvement in whom investigation has failed to reveal any specific diagnosis and who are then, because of the presence of such features as arthritis, myositis or Raynaud's phenomenon, categorized as suffering from a connective tissue disorder of unspecified type. It is evident that this usage is little more than a cloak for diagnostic uncertainty, and often an excuse for a trial of corticosteroid therapy.

When Sharp claimed in 1972 that some of these patients could be allocated, through their possession of antibodies to a Saline Extractable Nuclear antigen (ENA), to a new disease entity (Sharp *et al.*, 1971; Sharp *et al.*, 1972) widespread interest was aroused. He called this disease mixed connective tissue disease (MCTD) as it was clinically characterised by a mixture of features usually separately associated with scleroderma, polymyositis and systemic lupus erythematosus. Within a few years similar cases also possessing antibodies to ENA were described from many different centres (Hench *et al.*, 1975; Minkin & Rabhan, 1976; Bresnihan *et al.*, 1977; Wolfe *et al.*, 1977; Bennett & O'Connell 1980).

The original publication describing MCTD made four claims for this disease that have subsequently become contentious:

(1) That the syndrome was clinically identifiable by a particular group of features.
(2) That the presence of high titres of antibodies to ENA was a unique (and hence diagnostic) serological feature.
(3) That cerebral, pulmonary and renal involvement, and vasculitis did not occur.
(4) That the condition had a benign prognosis and responded to small doses of corticosteroids.

It was the last two of these claims that were the occasion for particular enthusiasm by physicians. Unfortunately this enthusiasm has needed to become somewhat tempered over the succeeding years, and these claims must be critically reviewed.

In 1972, MCTD was described as a clinical syndrome in which Raynaud's phenomenon, arthralgias, a mild arthritis (usually non-erosive, non-deforming), puffy hands, abnormal oesophageal mobility, myositis, and lymphadenopathy occurred in most patients. Hypergammaglobulinaemia occurred in 75% and anaemia and leukopenia in 50% (Table 1). Many of the relatively common features of SLE such as photosensitivity, alopecia and mouth ulcers were apparently rare in MCTD and rashes, serositis and myocardial involvement were described as uncommon. Clinically apparent renal,

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TABLE 1. Clinical characteristics of twenty-five patients with mixed connective tissue disease

Characteristic	Percentage
Arthritis/arthralgias	96
Swollen hands	88
Raynaud's phenomenon	84
Abnormal oesophageal motility	77
Myositis	72
Lymphadenopathy	68
Fever	32
Hepatomegaly	28
Serositis	24
Splenomegaly	21
Renal disease	40
Anaemia	48
Leukopaenia	52
Hypergammaglobulinaemia	80

Data taken from Sharp *et al.* (1972).

pulmonary and neurological disease were uniformly absent, and vasculitis had not been reported. The clinical picture of these patients resembled early scleroderma more than systemic lupus erythematosus and appeared to be confined to adults.

Since 1972 our conception of this disorder has evolved and undergone modification. Organ involvement is often more extensive than was previously thought, and in some series there has been a notable evidence of serious complications (Brodie *et al.*, 1977; Singsen *et al.*, 1977).

The condition has now been recognised in children (Singsen *et al.*, 1977; Peskett *et al.*, 1978; Baldassare *et al.*, 1978), the age of patients with MCTD ranging from 5–80 years with a mean of 37 years; 80% female. The actual prevalence is unknown but it would appear to be more common than polymyositis but less common than SLE. Renal disease, neurological disease, pulmonary disease, extensive gastrointestinal involvement, and vasculitis must also be added to the clinical picture, and a deforming erosive arthritis and pleomorphic skin changes are reported. It has also been recognised that there is an evolution of the disease with time (Sharp, 1979). In the early phases of the disease the patient may have only mild symptoms, such as Raynaud's phenomenon, arthralgias and myalgias, but may subsequently develop other features with major organ involvement. In Sharp's original series 75% of the patients had an arthritis, mostly of the non-deforming, non-erosive type. Only three of his patients had a rheumatoid-like arthritis. Several series (Halla & Hardin 1978; Ramos-Niembro *et al.*, 1979; O'Connell & Bennett, 1977) have since reported patients with a deforming and erosive arthritis which resembles rheumatoid arthritis, and which it has been suggested responds to anti-rheumatic drugs such as gold (Halla & Hardin 1978). The original dermatological features reported were Raynaud's phenomenon and puffy hands. Since then many others have been added. Sharp (1980) describes lupus-like rashes of the face including malar eruptions, non-scarring erythematous plaques and chronic scarred discoid plaques in up to 50% of cases. Less frequent findings (about 25%) included alopecia, vitiligo, photosensitivity and oral ulceration. Uncommon findings (10%) in his series were urticaria, telangiectasia and heliotrope lids. Bennett and O'Connell (1980) have recently added erythema nodosum, Lyell's disease and erythema multiforme to the list.

Pulmonary disease is now well documented (Singsen *et al.*, 1978; Parker *et al.*, 1978). In one study

(Harmon *et al.*, 1976) 80% of all patients had evidence of pulmonary disease on lung function tests. The most common findings were decreased diffusing capacity and reduced lung volumes; X-rays showed diffuse interstitial infiltrates and, less frequently, shrinking lungs or pleural disease. Pulmonary hypertension has also been reported (Rosenberg *et al.*, 1979) and fatalities in MCTD have been recorded in association with progressive pulmonary failure secondary to parenchymal lung disease and pulmonary hypertension (Jones *et al.*, 1978; Parker *et al.*, 1978).

Sharp & Anderson (1980) now estimates that about 10% of patients develop renal disease. This is often mild; however it may be the major problem and death can occur from progressive renal failure (Fuller *et al.*, 1977; Wolfe *et al.*, 1977). Renal biopsies performed on twenty patients taken from a series of over 300 cases of MCTD included five with mesangial hypercellularity, five with focal, three with membranous and one with membranoproliferative glomerulonephritis, and one patient had severe intimal proliferative vascular changes (Singsen *et al.*, 1977). Many cases of immune complex nephropathy have also been reported (Bennett & Spargo, 1977; Rao, Berkseth & Crosson, 1976; Fuller *et al.*, 1977). Neurological involvement is diverse. A high incidence of neuropsychiatric problems has been reported (Bennett & O'Connell, 1980); other manifestations have included trigeminal neuropathy, vascular headaches, seizures, peripheral neuropathies, cerebral infarction and haemorrhage, and aspectic meningitis. Sharp estimates that about 10% of patients have mild neurological abnormalities. Vasculitis, originally not recorded, is now thought to occur in about 2% of cases, and has been fatal (Cooke & Lurie, 1977). Abnormalities in oesophageal motility had been reported in 77% of the original cases and subsequently exclusive intestinal involvement similar to that seen in systemic sclerosis has been recorded (Norman & Fleischmann, 1978). Other findings which may occur in patients with MCTD include Sjögren's Syndrome (Alarcon-Segovia, 1976) and Hashimoto's thyroiditis (Wolfe *et al.*, 1977).

Many patients with MCTD fulfill the ARA criteria for either rheumatoid arthritis (Ropes *et al.*, 1959) or SLE (Trimble *et al.*, 1974, Cohen *et al.*, 1971) or both, and many have features regarded as characteristic of systemic sclerosis. Therefore the concept of MCTD as a distinct clinical entity has come under critical fire.

The initial impression that a diagnosis of MCTD implied a good prognosis and a favourable response to low dose steroids is also now questionable. The presence of renal disease, vasculitis, neurological involvement and pulmonary involvement, all of which have proved fatal, belie the original concept of a benign disease, and a guarded prognosis is appropriate certainly at first presentation (Brodie *et al.*, 1977; Sharp & Anderson 1980; Niemelstein *et al.*, 1980). Therapy is equally confusing. Many of these patients have mild disease and can be managed on low dose steroids, or even non-steroidal anti-inflammatory drugs alone. Patients with severe complications will obviously require intensive therapy which may involve the use of high dose steroids and immunosuppressive agents as in SLE and polymyositis.

The defining feature of this disease is a serological test. These patients have a high titre of antibody in their sera to a ribonuclease sensitive component of ENA which is a nuclear riboprotein antigen (RNP).

Work by Mattioli & Reichlin (1977) has resolved ENA into two parts, a soluble ribonucleoprotein and a non-nucleic acid protein designated Sm. Antibodies to Sm are restricted to patients with SLE (Notman, Kurata & Tan, 1975) whereas MCTD patients have antibodies only to RNP and these usually in high titre. RNP antibodies can be detected either by measuring the antibody titre by haemagglutination against the RNAase sensitive component of ENA (Sharp *et al.*, 1971; Sharp *et al.*, 1972) or more simply by immunodiffusion using appropriate prototype sera and thereby indentifying only antibody to RNP (Reichlin & Mattioli, 1972, Parker, 1973). Other serological abnormalities can occur in MCTD (Table 2). Most patients have high titres of fluorescent ANA which produces a speckled pattern, and high titres of rheumatoid factor are present in up to 50% (Sharp & Anderson,

TABLE 2. Laboratory findings in patients studied

Investigation	Percentage
Elevated ESR	100
Positive ANIF	100
Positive RNP antibodies	100
Negative dsDNA antibodies	100
Negative Sm antibodies	100
Anaemia	75
Leukopaenia	75
Elevated gamma globulins	75 (17)*
Elevated IgG	75 (9)
Positive ss DNA antibodies	65
Positive direct Coombs test	60 (11)
Elevated CPK	43 (18)
Reduced C_4	30 (19)
Positive latex fixation	25
Positive SCAT	25
False positive VDRL	10

* Numbers in parentheses indicate that only that number of of patients, out of the total twenty, underwent that particular investigation.

Data taken from Bennett and O'Connell (1980).

1980). Serum complement levels are reduced in about 25% (Wolfe *et al.*, 1977) and immune complexes have been reported (Parker, 1977; Ibanes de Kasep, Sturgein & Agnello, 1977). They may also have antibodies to DNA, usually in lower titres than in SLE (Wolfe *et al.*, 1977).

The problem remains as to whether MCTD being essentially serologically defined warrants the status of an independent disease, as its clinical features and natural history have been difficult to unravel from SLE, polymyositis and systemic sclerosis.

Antibodies to RNP are not confined to MCTD but are found in SLE, R.A., polymyositis and other rheumatic disorders (Parker, 1973; Sharp *et al.*, 1976; Maddison, Mogovero & Reichlin, 1978). Reichlin has suggested that patients with MCTD have a more benign form of SLE in which RNP may play a protective role. To support this suggestion he cites the low incidence of renal disease in patients with RNP antibodies (Reichlin & Mattiolo, 1972; Gaudreau *et al.*, 1978) and ENA which is known to interact with DNA (Hamburger, Friedlander & Borland, 1974) may prevent the formation of DNA/anti-DNA immune complexes and, therefore, subsequent tissue damage. There are also patients who, although possessing clinical features suggestive of classical MCTD, are ENA negative. How are such patients to be classified? The passage of time usually provides the answer and these ENA negative patients often develop one of the classic, well categorized rheumatic diseases.

It is with these arguments in mind that the suggestion by Christian (1980) and Le Roy, Maricq & Kahaleh, (1980), that caution should be employed before abitrary classification and categorization of mixed connective tissue disease is made, is attractive. Christian argues that it is only in a statistical sense that prognosis and management are influenced by diagnosis whether typical or overlap, and it is the pattern of organ involvement that gives a more accurate guide. As a group, MCTD patients with high titres of antiRNP antibodies and no antibodies to DNA are less likely to show renal or nervous system involvement than are patients with typical SLE, but in individual patients with the mixed syndrome serious systemic complications may occur and the prognosis is altered.

His strongest argument for analysis of mixed connective tissue syndromes relates to aetiology and pathogenesis. The atypical and overlap syndromes may reflect variable host response to common aetiological factors. Antibodies to RNP are maximally expressed in a mixed pattern of disease but are also associated with well characterized rheumatic diseases. The detailed study of patients with MCTD may provide particular opportunity to characterize aetiological and pathogenetic factors that are common to other connective tissue disorders.

Le Roy *et al.* (1980) suggest that these non-conforming patients might be more realistically called undifferentiated connective tissue disease (UCTD) until such time as diagnostic features declare themselves. He argues that introduction of a 'new' disorder with clinical features derived from a number of other diseases, and which has no unique pathognomonic characteristics, is confusing and that UCTD is a more useful and flexible concept than mixed or overlapping syndrome. The debate continues but the onus for providing their case still rests with the proponents of MCTD and recent re-evaluation of twenty-two of Sharp's original twenty-five patients would suggest that patients with MCTD do not eventually behave very differently from patients with the classic diagnostic features of SLE, myositis or PSS, (Neimelstein *et al.*, 1980).

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Huber's stain for resin embedded cutaneous tissue: a modification

SIR, Drs Chu, Smith and MacDonald were disappointed by the results of the Huber stain for resin embedded tissue. If used in its original form it certainly is a difficult procedure with a variable quality of staining. But if successful it also offers beautifully contrasting colours which are often superior to those of other stains. Because of its usefulness in cytomorphological research we attempted to improve the method (with success) as demonstrated in our study of mycosis fungoides (van der Putte & van der Meer, 1981). We had to extend staining time but gained considerably in colour contrast.

Biopsies are fixed in 2% glutaraldehyde or Karnovsky fixative, postfixed in 1% osmium tetroxide, dehydrated in alcohol and propyleneoxide and embedded in Epon 812 by standard techniques. Semithin 0·55–1 μm sections are cut and placed on clean glass slides. These are kept overnight at a temperature of 70°C to prevent detachment of sections in the alkaline methylene blue.

Staining reagents

Solution A, 0·01% basic fuchsin (Chroma 1A308) (diluted from a solution of 0·1% which can be kept in stock for at least a month). Solution B, 0·5% methylene blue (C.1.52015). Solution C, 0·1 N NaOH. All solutions are prepared shortly before use.

Staining procedure

Slides are placed in a jar with solution A for 10–90 s at 80°C, followed by distilled water for 1 min at 80°C and then dried thoroughly. The slides are next placed in a jar with solution C for 1 min and subsequently in a mixture of solutions B and C (1:1) for about 30–120 s. The excess of methylene blue is removed in distilled water. The sections are dried for some hours and mounted in malinol (Chroma 3c242).

Staining times often vary for different specimens, which is probably due to slight differences in the texture of the plastic. Dried rings of stain no longer cause any trouble because of the use of jars and a far lower concentration of the basic fuchsin.

Results

Cytoplasm in diverse shades of pink, mitochondria darker pink, phagocytosed material red, nuclear heterochromatin dark blue, nucleoli bright red, granules of mast cells bright red and of eosinophils green. Collagen fibres light blue, elastic tissue purplen.

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Book Reviews

Contact Dermatitis. ETAIN CRONIN (1980) Edinburgh: Churchill Livingstone. Pp. 915. Price £28.00.

It is not often that one is called on to review a one-author book that is a landmark of its kind. There is no doubt that this is such an occasion. One thinks back to 1915, when Prosser White published his remarkable 'Occupational Affections of the Skin'. It has taken 65 years for a comparable volume to emerge. We have, as a country, lagged a little behind our colleagues in Europe in publicizing our interest in contact dermatitis and it is very fitting that Etain Cronin has taken on the tremendous task of distilling the world-wide experience of this subject in a British context. No one in this country has been more dedicated to the subject of which she writes and endowed with the balanced judgement to assess the value of the voluminous literature that she quotes. It must have been a tremendous task. This book certainly provides the most comprehensive reference available to all aspects of the subject. Though concisely (and at times tersely) written, its very size is an indication of the enormous growth of interest in the subject in the last four decades. Nothing of importance is omitted, and the whole book is pervaded by that very necessary quality (lest one be carried away by mirages), that of commonsense. The difficulty of distinguishing irritant from allergic reactions, e.g. in testing quaternary ammonium substances, is fully accepted. In her particular spheres of interest, dyes, textiles, cosmetics and metals, Dr Cronin speaks with a particularly authoritative voice. In the section on woods and plants she has had the invaluable assistance of Dr John Mitchell, and it is pleasant to see the author acknowledge the help given by her clinic sister. Where would all of us be without these experienced and knowledgeable persons? She also gives credit to Drs Calnan and Meara for their prescience in setting up the Contact Clinic at St John's Hospital for Diseases of the Skin; we all owe them a great deal. I also find it particularly pleasant to see acknowledged the help and contribution given by industrial and pharmaceutical firms in providing information so freely in a combined attempt to elucidate difficult problems. This does not occur everywhere and is a relationship that we should cherish.

What of the criticism? No book is perfect. Well, I dislike the thick paper. Will the spine stand the constant opening to which a reference book of this type will be subject? The more so because the references to a particular agent are often scattered over many pages; it takes a little time, for instance, to locate rubber chemicals as a cause of local depigmentation. This brings me to the index, which I think is inadequate. It is a common complaint nowadays. I yearn, personally, for a return (at least some way) to the marvellous and comprehensive index of Prosser White which, in my 4th edition, constituted 40% of the text (and almost eliminated the need for this, except as a reference point or footnote).

Finally, may I make a plea for the inclusion of even more epidemiological comparisons of allergic reactions in different geographical and cultural settings. The vast experience Dr Cronin has drawn from the St John's Clinic is the finest in the country. But this is going to be an international 'Bible' of its subject and the rather selective material sent to St John's might be balanced, perhaps, by an assessment of the experience of other centres in tabular or diagramatic forms.

These are relatively minor criticisms. This book is a landmark in British dermatology and will be, if it has not already become, an indispensable working book for everyone dealing with dermatological patients, whether they actively practice extensive patch testing or not. For those who do, it will be a constant source of reference, and for those who do not, it will be an Aladdin's cave.

D.S.WILKINSON

Allergic Diseases. Diagnosis and Management. Edited by Roy Patterson (1980) Philadelphia and Toronto: J.B.Lippincott Co. Distributed in U.K. by Harper and Row, London. Pp. 714. Price £28.00.

This is a second edition of a book containing contributions by twenty-eight authors, mostly faculty members of the Allergy and Immunology Section of the Department of Medicine at Northwestern University Medical Centre in Chicago. The first chapter of the book comprises as excellent and succinct a summary of human immunology as can be found anywhere. It is followed by a series of chapters on immediate hypersensitivity. Important airborne allergens are then reviewed with special emphasis on pollens; the geographical bias reduces the value of this review to those working outside North America. Individual diseases for which there is evidence of an allergic basis are then individually considered, and there are in addition several chapters on special topics such as 'Corticosteroids in the treatment of allergic diseases', 'Eosinophilia' and 'Pruritus', and an unusually comprehensive review of the problem of drug allergy.

As one would expect in a multi-author volume of this size and scope, there is considerable variation in the quality of individual chapters, and this inevitably makes it unfair to indulge in generalizations in its criticism. I found much of the book lacking in sparkle, due to the failure of authors to impart personal flair to their own chapters. One seeks guidance through the morass of available information, and this was largely missing. Rigid impartiality is correct form in a scientific paper but out of place and tedious in a review.

In a book like this there will be subjects of which one has a firm grasp and others of which one does not. The clinical information contained in the chapter on atopic eczema was very misleading and, in places, frankly incorrect. After reading this chapter I found myself worrying about those other statements one had accepted unquestionally in chapters whose subject matter was less familiar.

These criticisms apart, there is much to praise in this book and I certainly learned much from certain chapters. At £28 it is inexpensive and, in a perfect world, every dermatologist would own it. I would not strongly recommend that dermatologists go out and buy this book, as much of it is of dubious relevance to the practice of the speciality, and that which is relevant is on the whole better dealt with in dermatological texts.

D.J.Atherton

The Nail in Health and Disease. Nardo Zaias (1980) Lancaster: MTP Press Ltd. Pp. 260. Illustrated. Price £35.00.

Scientists and clinicians interested in the nail cannot fail to have noted and appreciated Dr Zaias's enormous contribution in the field during the last 20 years; it is difficult to recall a single publication from his unit that has been anything but stimulating and original. His book has been awaited with a great sense of anticipation that it would inevitably be the definitive work on the subject.

Unfortunately, although this volume is an important work, as a book it is very disappointing. It is intended to 'Familiarize the medical practitioner with the nail unit in a way which will render, correctly and more easily the diagnosis of nail diseases. At the same time it serves to encourage the treatment and corrective measures of the abnormalities, if possible, based on anatomical and physiological knowledge. . . . It is also the intent of the author to compile as many facts about human as well as animal nails and claws so that this may be used as a reference text.' Contained within the 260 pages is all the material needed to achieve these prefatorial aims, but the skeleton does not make a working body.

The English is styled like a reference book, succinct but often perfunctory, and studded with many numbered references. It is certainly not a comprehensive reference book, presumably because of the limitations of size and cost; neither is it a ready volume for the clinician needing a diagnostic office manual. Both of these aims could have been satisfied if less space had been used for figures; more than eighty pages contain only diagrams and clinical pictures mostly of poor quality and often needlessly repetitive and large. Figures are essential in a visual subject such as nail diseases, but bad ones are counter productive. Dr Zaias has purposely set out to exclude controversial reference material but this had led to many omissions. Controversy and differences of opinion are ever present in clinical medicine; it is in these areas that the uninitiated need the opinions of wise and experienced observers.

This book is a significant contribution in a field in which there is a sparcity of collected material. The criticisms outlined above must be weighed against the very high standards set by the author in his published works. One hopes that a modified second edition will appear in the not too distant future.

R.P.R.DAWBER

British National Formulary 1981 Number 1. (1981) London: British Medical Association, The Pharmaceutical Press. Pp. 387. Price £3.80.

Those who set out to change the Establishment rightly feel that they must justify their actions. And this is a change. The previous eleven issues of that well-loved and much-thumbed 'B.N.F.' had been with us for 35 years and it was, in every sense, an indispensable pocket guide to prescribing. In their preface to this edition, the Editorial Committee explain that the change of format has been in response to a request for 'more guidance for prescribers on the choice . . . available for the treatment of a particular disease or condition'. This is a logical extension of the aims of previous editors and is met by developing the guidelines introducing each group of drugs and by the inclusion of several valuable new sections concerned with the effect of hepatic and renal disease on drug metabolism. The drugs and applications are arranged partly by their mode of action and partly under headings referring to the disease for which they are commonly prescribed, e.g. preparations for psoriasis and eczema follow a comprehensive list of the topical corticosteroids. This is probably a sensible empirical approach, though it leads to a few gaps and anomalies. Thus, dapsone only appears as an anti-leprotic and there is no mention of methotrexate for psoriasis. To the dermatologist this matters little but the general practitioner may be puzzled. The list of skin preparations is comprehensive, though some items are difficult to find; antibacterials alone are cited in one place but in combination with hydrocortisone, etc., they appear in the 'compound' skin preparations list. Perhaps sensing this problem, the editors have mercifully provided a superlative index.

The most controversial feature of the new edition lies in the inclusion of virtually all the current proprietary preparations available (and a few I have never heard of) and an indication of the relative price range of everything listed in the book. This is based on 'the need to make the best use of available resources' but is bound to give rise to misgivings in some quarters. It is manifestly difficult to avoid the impression of penalizing new drugs and topical agents of value that have to absorb initially the high cost of development and research; we must hope that this does not deter those firms that encourage such research.

The Formulary is beautifully produced, with clear and attractive typeset. Unfortunately, its 387 pages convert it into a 'white coat pocket book' only and the prospect of the two promised issues each year, to keep up with changes in the proprietary field, is daunting.

But the more one dips into this New Look Formulary, the more impressed one becomes by the skill, expertise and judgement of the Editorial Committee. They ask us for constructive criticisms; we should do what we can to help them.

D.S.WILKINSON

Hautnebenwirkungen interner Arzneimittel. Eine kommentierte Synopsis der heutigen Medikamente. Cutaneous Side Effects of Systemic Drugs. A Commentated Synopsis of Today's Drugs. ZASPAR ZURCHER and ALFRED KREBS. Basel: S. Karger. Pp. 370, 56 Tables. Price: SFr. 123.00, US $73.75

This is a very useful reference text on drug eruptions. Written by a Swiss physician and a Swiss professor of dermatology it is a massive compilation from the literature (thousands of references) of all drug eruptions reported in a large number of journals, text-books and specialized monographs over many years. The general layout is rather different from other monographs covering the same subject. The main message is conveyed in huge tables allowing one to see at a glance for example which antihistamines have been reported to cause fixed drug eruptions, urticaria or light sensitivity. There is also a good attempt to indicate the reported frequencies of the various manifestations, but inevitably little attempt to assess the validity of the original reports. There is no pretence to discuss the mechanisms of drug eruptions.

Some parts of the text and the tables are summarized in English but only a very modest understanding of German is necessary to fine one's way about the book once the initially slightly daunting basic layout has been understood.

R.H.CHAMPION.

Meyler's Side Effects of Drugs. Edited by M.N.G.DUKES (1980) Amsterdam: Excerpta Medica. Ninth Edition. Pp. 859.

Meyler's Side Effects of Drugs has established itself as a most valuable contribution ever since its relatively slender 1st edition of 1957. The 9th edition, like the 8th of 1975, has been edited by Dr Dukes with forty-two contributors mainly from Europe and Canada. However, it is not just a simple update of the previous editions. It has now become more of a comprehensive textbook on drug reactions. It leaves the minutiae and unconfirmed or isolated case reports to the Side Effects of Drugs Annual, also edited by Dr Dukes, and to other more massive and less accessible reference publications. In consequence the book is now more readable, more authoritative and, yes, 24 percent smaller. For example the 'drugs used on the skin' chapter has diminished from 76 pages with 675 references to 30 pages with 166 references. Here we find a full account of systemic and local effects of topically applied drugs, including a list of patch test concentrations.

Details of the various patterns of drug eruption appear scattered throughout the text, often providing slightly less detail than makes it ideal for dermatologists. Nevertheless we have 800 pages of closely knit information, with four different and very comprehensive indexes. Any doctor will need to have access to this book, even if only in the hospital pharmacy.

R.H.CHAMPION

Manual of Contact Dermatitis. Second Edition. S.FREGERT (1981) Copenhagen: Munksgaard. Pp. 139. Price £11.35.

I had already bought a copy of this little book before I was sent it to review. I think it is a brilliant distillation of the main ideas about contact dermatitis and patch testing arrived at by the International Contact Dermatitis Research Group and the North American Contact Dermatitis Group. At the same time it bears the unmistakable stamp of its incisive author.

It is written so concisely that an enormous amount of information and concepts are included between its covers and there are enough new points to make it worth reading even if one is familiar with the original edition. What is more, as the author states in his preface, it is written mainly for dermatologists who are not specialists in contact dermatitis.

Its small size makes it truly a manual, a rare distinction among 'manuals' these days. I would recommend anyone reading this review to buy it, read it and then read it again. It is a pocket course in contact dermatitis.

R.J.G.RYCROFT

Selected Methods in Cellular Immunology. Edited by BARBARA N.MISHELL and STANLEY M.SHIIGI. Oxford: W.H. Freeman and Company. Pp. 486. Price £17.70.

This is a reference book of methods to prepare and examine cells for immunological research, and though the techniques refer mainly to the preparation of mouse cells, including a chapter on their separation, the information is relevant to examination of clinical problems.

The book reflects its intention as a manual for teaching students and research scientists. Many techniques are described for preparation of suspensions of separated cells, the preparation and use of antisera to detect cellular antigens, and induction of antibody synthesis *in vitro*. Most information concerns the lymphoid cells, and there is no reference to mast cells and basophils which are the essential cells in anaphylaxis, and probably participate in delayed hypersensitivity, and no descriptions of bone marrow cell cultures with feeder layers. The minor details of technique are valid and helpful, but it is to be regretted that there are no hints on how to overcome troubles. Who, on occasion, has not found a carefully prepared suspension of cells to aggregate spontaneously and needs to separate them without damage?

The reviewer concludes that this carefully prepared description of selected methods for tests, mainly on lymphoid cells, is of value as a reference book in the library of research laboratories.

W.E.PARISH

Lasers in Photomedicine and Photobiology. Proceedings of the European Physical Society Quantum Electronics Division Conference. Florence, Italy. September 3–6, 1979. Edited by R. PRATESI and C.A.SACCHI (1980) Berlin: Springer-Verlag. Pp. XIII, 235, 108 Figures, 20 Tables. Price approx. US$29.50.

Leon Goldman recently wrote a slight review of the use of lasers in Dermatology (*International Journal of Dermatology*, **19**, 329–331, 1980) and it would seem that advances from his pioneering studies have been rather limited. With contributors such as Parrish, van der Leun, Magnus, Tronnier and Szabo, a book with the above title might be expected to correct such an impression. However, the editors, in their preface, make it clear that the conference was not intended for physicians, surgeons or, for that matter, a number of associated researchers. One must assume that it was for the benefit of physicists and the title may be misleading. Only the last two sections, devoted to spectroscopy of various kinds, illustrate the extensive use of lasers in important and interesting studies which are of basic photobiological content. For the uninitiated, these are difficult to understand. The rest of the book, a long introduction followed by sections on Photodynamic Therapy of Tumours, Photodermatology and Phototherapy of Hyperbilirubinemia, deals primarily with background material of conventional source photobiology and photochemistry, with suggested applications for laser use and little bits of actual laser work thrown in. Included in the Photodermatology section are preliminary reports by Anderson and Parrish on various UV-laser effects on human and experimental animal skin and by Garcia and Szabo on Helium-Cadmium laser (325 nm) effects on dermal nerve fibres. In addition, there is a report by Anders and her colleagues on the Applications of Dye Lasers in Photodermatology, in which certain of the basic concepts are almost incomprehensible according to received knowledge. This is a pity because this group appear to have done a great deal of work. Two concluding points. First, the editors confess that not all the printed papers were unanimously accepted for publication. Secondly, we wait until page 115 before there is any mention of Leon Goldman.

B.F.JOHNSON

Year Book of Dermatology 1980. Edited by RICHARD L. DOBSON and BRUCE H. THIERS. Chicago, London: Year Book Medical Publishers, Inc. Pp. 407. Price £25.50.

This flourishing, hardy annual serves three purposes. It enables dermatologists to cover a wide field of journals, it reminds readers of articles read and forgotten, and thirdly, if one buys each volume, it is a quick way of gaining entry to the recent literature of the subject. Does it succeed? I think it does, although obviously summaries, however good, are no substitute for reading and personally evaluating the original articles despite the succinct editorial comments.

Can it be improved? One suggestion is to include more abstracts of papers of quality from journals written in languages other than English.

The introductory article this year is on HLA, Ia and the skin, and I can recommend this as a good account of this expanding field. There is also a current literature quiz which serves as a quick personal way of showing how essential the Year Book is becoming.

N.R.ROWELL

British Journal of Dermatology (1981) **104**, 729.

Editorial

This issue of the Journal is the last that will be the responsibility of the present editorial team. The new editor, although by no means new to the Journal, is Dr J.L.Burton (to whom correspondence and papers should in future be directed).

The editing of the journal has been based on Cambridge for the past 13 years, and it is appropriate here to give due credit to Dr Arthur Rook who was editor for the first half of this time. The Journal is indeed very different now to what it was in the '50s and '60s, larger in size, more investigative as well as clinical in its scope, more papers from abroad (about 60 percent) and with a new publisher. It is appreciated that not all papers are read by all readers. The balance of papers reflects the balance of those submitted rather than any deliberate editorial policies.

At this time many very distinguished members of the editorial board and some of the assistant editors, who have been part of the team for over a decade, are also retiring from the Journal's activities. Only a small minority of papers can be refereed by the editorial board but their active assessments and availability 'for a rainy day' greatly merit our thanks. Public tribute should also be paid to the 200 or more anonymous referees who have given so much time and effort in refereeing virtually every paper submitted. 'The strength of a journal rests with its referees'—as was pointed out by one author who disagreed with some criticism of a paper! Other invaluable but anonymous and honorary workers behind the scenes have been the late Professor J.Milne, and subsequently Dr H.R.Vickers, who have prepared the Index; Dr J.M.Beare and Dr R.Dawber, who have collected the curricula vitae for the News and Notices; Dr J.N.S.Mitchell and then Dr A.Highet for the time consuming and tedious task of checking proofs. Blackwells have provided every facility and co-operation. Most importantly my secretary Mrs Anita Mead has been personally and unstintingly responsible for dealing with over 3,000 papers, letters reflected in a postage bill of £500 a year and an estimated three tons of paper!

We would all like to wish Dr Burton every success and joy with the Journal, knowing very well that it is in the best of hands.

R.H.C.

British Journal of Dermatology (1981) **104**, 730.

News and Notices

2nd Dermatopathology Course, Rome, Italy, 17–18 July, 1981

Under the sponsorship and organization of the Foundation for Research in Dermatology an intensive two day course on cutaneous neoplasms will be held in the Catholic University, Rome, Italy, on Friday 17 and Saturday 18 July 1981.

The Course will be given by Dr A.B. Ackerman, Professor of Dermatology and Pathology, and Director of Dermatopathology, New York University School of Medicine.

Dr Ackerman will concentrate on words, terms and phrases to avoid in the language of neoplasia; criteria for predicting biological behaviour from histological patterns; diagnosis of neoplasms by pattern analysis; melanocytic naevi and malignant melanomas; keratoacanthoma; spindle cell neoplasms; cysts; and regression of neoplasms.

Enrolment is limited: Fees: US$100 (before 30 May), US $120 (after 30 May).

Enquiries and requests for registration and hotel reservation forms should be directed to Ernestine Shargool, Secretary of the Foundation for Research in Dermatology, Largo Gemelli 8, 00168 Rome, (tel.: 06-3385451).

Besides hotel accommodation a limited number of rooms at moderate prices will be available in Religious Institutions. For accommodation please contact the American Express Company, Piazza di Spagna 38, 00187 Rome (tel.: 06-67641).

Practical Skin Pathology Course

A 40-hour, AMA, AAD, ASCP-approved course in Practical Skin Pathology will be given at Grossinger's, Grossinger, New York, 30 August–4 September 1981. This is a rigorous, comprehensive and practical review and update of dermatopathology for pathologists and dermatologists. Microscopes and a two volume syllabus will be provided. There will be twenty-nine formal lectures, eight supervised microscope sessions of 400 unknown workshop slides and additional time for optional independent study of microscopic slides. The course directors are Drs M.Brownstein and L.Golitz. The guest faculty includes Dr A.Ackerman, J.Bhawan, M.Black, J.Maize, A.Mehregan and B.Michel. The tuition is $400 ($200 for those in training). For further information, please write to Martin H.Brownstein, M.D., Skin Pathology Course, 2 Jordan Drive, Great Neck, New York 11021 or phone (516) 829-8578.

XVI International Congress of Dermatology 23–28 May 1982, Tokyo—Scientific Programme

Special Lectures

(1) Viral oncogenesis. (2) Prostaglandins in inflammation. (3) IgE in inflammation. (4) Epidermal differentiation. (5) HLA.

Advances in Dermatology

(1) Treatment of psoriasis. (2) Atopy. (3) Mycosis fungoides. (4) New drug eruptions. (5) Pruritus. (6) Lichenoid eruptions. (7) Acne. (8) Nail diseases. (9) Pseudolymphoma. (10) Pustular dermatoses (11) Contact dermatitis. (12) Alopecia. (13) Photochemotherapy. (14) Skin microbiology. (15) Dermatitis herpetiformis. (16) Immune vasculitis. (17) Diseases of the mucous membranes. (18) Deep mycoses. (19) Urticaria. (20) Virus diseases. (21) Mixed connective tissue disease. (22) Immunofluorescence in dermatology. (23) Histiocytosis. (24) Serology of syphilis. (25) Photodermatoses.

Symposia

(1) Dermatopharmacology. (2) Contact dermatitis. (3) Urticaria. (4) Bullous diseases. (5) Psoriasis. (6) Vasculitis. (7) Lupus erythematosus and related diseases. (8) Scleroderma. (9) Occupational dermatoses. (10) Malignant melanoma. (11) Malignant lymphomas of the skin. (12) Syphilis. (13) Viral diseases of the skin. (14) Psoralen phototherapy. (15) Atopy. (16) Photomedicine. (17) Porphyrias. (18) Physical therapy in dermatology. (19) Dermatitis herpetiformis and overlap cases. (20) Cutaneous drug reactions. (21) Retinoids. (22) Sexually transmitted diseases (excluding syphilis). (23) Ichthyoses. (24) Acne. (25) Dermatological surgery and cryotherapy. (26) Hair in health and disease. (27) New dermatoses. (28) Leprosy.

Workshops

(1) Pathogenesis of acne. (2) Mechanisms of ageing of skin. (3) Research in biophysical properties of skin. (4) Microcirculation and lymphatics. (5) Epidermal basement membrane. (6) Controversial issues on bullous disorders. (7) Viral carcinogenesis. (8) Epidermal cell + organ culture. (9) Culture of pigment cells. (10) Epidermal cell kinetics. (11) Collagen. (12) Advances in the pathogenesis of contact dermatitis. (13) Mechanisms in graft vs. host of skin. (14) HLA and skin disease. (15) Immunological mechanisms in dermatomycosis. (16) IF techniques and immunoelectronmicroscopy. (17) Complement and skin disease. (18) Immunological phenomena in psoriasis. (19) Langerhans cells. (20) Fibrous proteins and prekeratin. (21) Identification of cells in dermal infiltrates. (22) Interactions of microorganisms of skin. (23) Immunological and chemical approaches to treatment of melanoma. (24) Melanogenesis. (25) Epidermal growth factor. (26) Prostaglandins. (27) Skin proteases. (28) Chemotaxis. (29) Sun sensitivity and sun protection. (30) Mechanisms in photochemotherapy. (31) Structural and functional modulations induced by retinoids. (32) Scleroderma, subsets and classification. (33) Chlamydia infections. (34) Dithranol (anthralin) and related antipsoriatic compounds. (35) Anti-androgens and the skin. (36) Metabolism of drugs by skin. (37) EM methods. (38) DNA repair. (39) Percutaneous absorption. (40) T cell subsets.

Courses

(1) Structure and functions of the skin (6 hours). (2) Dermatopathology (6 hours). (3) Pigmentation (6 hours). (4) Paediatric dermatology (6 hours). (5) Immunodermatology (6 hours). (6) Photobiology, science and clinic (6 hours). (7) Tropical dermatology (6 hours). (8) Cutaneous surgery (6 hours). (9) Bacterial infections of the skin (3 hours). (10) Genodermatoses (3 hours). (11) Cytostatic and immunosuppressive therapy (3 hours). (12) Cryotherapy (3 hours).

In addition, there will be Case Presentations, Free Communications, Informal Discussions and Posters as previously announced. Dermatologists wishing to participate in these events are requested to contact the respective organizers: Dr D.S.Wilkinson, Whitecroft, Hervines Rd, Amersham HP6 5HT, England for Case Presentations; Professor F. Serri, Department of Dermatology, Universita Cattolica del Sacre Cuore, Via della Pineta Sacchetti 644, I-00168 Roma, Italy for Free Communications; Professor Dominguez Soto, Hospital General, Dr Manuel Geo Gonzalez, Calz de Tlalpan 4.800, Mexico 22 D.F. for Informal Discussions; Professor Alejandro Cordero, Carlos Pellegrini 1560, 1011 Buenos Aires, Argentine for the Posters.

APPOINTMENT

Dr Michael Beck has recently been appointed as Consultant Dermatologist to the Bolton and Wigan Area Health Authorities and Salford Area Health Authority (T).

He was educated at Sandbach School, Cheshire, and qualified in medicine in 1972 at Liverpool University, where he first developed an interest in dermatology during the undergraduate course.

He has worked at the Manchester Skin Hospital since obtaining his M.R.C.P. (U.K.) in 1977, during which time he has done research into percutaneous absorption from topical lead-containing medicaments, chronic urticaria and dithranol therapy. He has a number of publications, mainly reflecting his clinical interest in dermatology. He has more recently been actively engaged in contact dermatitis investigation, including the starting of a pilot study on the computerization of patch test results for the British Contact Dermatitis Group. The new post will include responsibility for contact dermatitis investigation at the Manchester Skin Hospital.

Index

SUBJECTS

- Acid α-naphthyl acetate esterase as marker of T lymphocytes, 31
- Acid glycosidases in basal cell epithelioma and seborrhoeic keratosis, 147
- Acne induced by dantrolene, 465
- Acrodermatitis enteropathica and zinc deficiency, 459
- Adenosine 5′-monophosphate in the treatment of herpes zoster (Correspondence), 351
- Albumin in extensive skin disease, 389
- Allergy to penicillin and repeated patch testing, 157
- Alopecia areata regrowth induced by *Primula obconica*, 339
- Alopecia areata treated unsuccessfully with dapsone, 597
- Alopecia areata treated with zinc sulphate, 483
- Angioimmunoblastic lymphadenopathy, skin lesions in, 131
- Antigens recall retarded (Summary), 101
- Argyria, occupational, 19
- Aromatic retinoid, Tigason, in Darier's disease, 675
- Aryl hydrocarbon hydroxylase activity in psoriasis (Correspondence), 352
- Aryl hydrocarbon hydroxylase activity in psoriasis (Correspondence), 353
- Arylsulphatases in tumours (Correspondence), 221
- Arylsulphatases in tumours (Correspondence), 222
- Atrophy from corticosteroids and the Duhring chamber, 57
- Autoantibodies to epidermal protease inhibitor, 621
- Autoradiography in cell kinetics, 277

- Basal cell epithelioma, acid glycosidases in, 147
- Behçet's disease treated by colchicine, 67
- Biochemistry. Acid glycosidases in basal cell epithelioma and seborrhoeic keratosis, 147
- Biochemistry. Characterization of tyrosinase inhibitors, 531
- Biochemistry. Distribution and degradation of albumin in extensive skin disease, 389
- Biochemistry. Human dermal microvasculature: II. Enzyme histochemical and cytochemical study, 521
- Biochemistry. Incorporation of D-(^{3}H) glucosamine into normal and psoriatic epidermal glycoconjugates, 429
- Biochemistry. Sulphhydryl and disulphide staining of subepidermal hyaline bodies, 437
- Biochemistry. Tyrosinase-rich GERL in melanin synthesizing cells, 169
- Biological ageing in human epidermis, 627
- Body, image, cutaneous, 611
- Book reviews:
  - *A Colour Atlas and Textbook of the Histopathology of Mycotic Diseases.* Francis W.Chandler, William Kaplan and Libero Ajello, 489
  - *A Colour Atlas of Hand Conditions.* Bruce W. Conolly, 112
  - *A Guide to Leprosy Control.* World Health Organization, 490
  - *A Handbook of Medical Hypnosis.* Fourth Edition. Gordon Ambrose and George Newbold, 357
  - *Allergic Diseases. Diagnosis and Management.* Edited by Roy Patterson, 724
  - *Basic and Diagnostic Aspects of Cutaneous T Cell Lymphomas.* E.M.Van Der Loo, 225
  - *Biological Effects of Ultraviolet Radiation.* Walter Harm, 490
  - *British National Formulary 1981 Number 1*, 725
  - *Clinical Practice in Sexually Transmissible Diseases* D.H.H.Robertson, A.McMillan and H. Young, 111
  - *Collagenase in Normal and Pathological Connective Tissues.* David E.Woolley and John M. Evanson, 112
  - *Common Skin Disorders. A Manual for Physicians and Patients.* Ernest Epstein, 355
  - *Contact Dermatitis.* Etain Cronin, 723
  - *Cutaneous Side Effecte of Systemic Drugs A Commentated Synopsis of Today's Drugs.* Z. Zurcher and A.Krebs, 726
  - *Cytodiagnosis in Dermatology*, V.Ruocco, 226
  - *Dermatologia.* J.A.Esteves, A.P.Baptista and F.G. Rodrigo, 113
  - *Dermatology.* G.K.Steigleder and H.I.Maibach, 609
  - *Diseases of the External Ear. A Otologic-Dermatologic Manual.* B.H.Senturia, M.D.Marcus and F.E.Lucante, 225
  - *Entomology in Human and Animal Health.* 7th edn. R.F.Harwood and M.T.James, 224
  - *Haar und Haarkrankheiten.* Edited by C.E.Orfancs, 609
  - *Hormone Therapy of Acne. Clinical and Experimental Principles.* Doris Fanta, 223
  - *Lasers in Photomedicine and Photobiology.* Edited by R.Pratesi and ClA.Sacchi, 727
  - *Laser Treatment for Naevi.* Koshio Ohshiro, 356
  - *Maladie de Bowen. Erythroplasie Carcinome in Situ.* P.Jeandidier and J.Beurey, 357
  - *Manual of Contact Dermatitis.* S.Fregert, 726
  - *Manual of Dermatology*, J.P.Callen, M.A.Stawiski and J.J.Voorhees, 489
  - *Manual of Skin Diseases.* Gordon C.Sauer, 223
  - *Meyler's Side Effects of Drugs.* Edited by M.N.G. Dukes, 726
  - *Paraneoplasia: Biological Signals in the Diagnosis of Cancer*, Jan G.Waldenström, 224
  - *Photochemotherapy. Information for Doctors and Patients.* Gerhard Weber, 226

  Recent Advances in Dermatology—5. Edited by Arthur Rook and John Savin, 355
  Selected Methods in Cellular Immunology. Edited by B.N.Mishell and S.M.Shiigi, 727
  The Nail in Health and Disease. Nardo Zaiae, 724
  The Physiology and Pathophysiology of the Skin. Edited by A.J.Jarrett, Volume 6, 490.
  Vasculitis. Edited by Klaus Wolff and Richard K. Winkelmann, 356
  Venereal Diseases. Ambrose King, Claude Nicol and Philip Rodin, 491
  Venereology in Practice. The Sexually Committed Diseases. By Niels Hjorth, Henning Schmidt and Howard I.Maibach, 111
  Year Book of Dermatology 1980. Edited by R.L. and B.H.Thiers, 720
Bullous pemphigoid and systemic lupus erythematosus, 71
17-Butyrate esters of hydrocortisone and clobetasone compared, 179

Carcinogenesis, early changes in skin, 485
Cell kinetics in psoriasis, 271
Cell kinetics of mouse skin by autoradiography, 277
Cerebellar ataxia and sensory neuropathy in dermatitis herpetiformis, 593
Cerebriform tongue, a sign of pemphigus vegetans, 587
Ceroid-lipofuscinosis, ultrastructure, 581
Clioquinol and neomycin allergy (Summary), 100
Clobetasone and 17-butyrate esters of hydrocortisone compared, 179
Colcemid and vincristine to measure proliferative rates in epidermis, 243
Colchicine in the treatment of Behçet's disease, 67
Cold reflex urticaria, 83
Cold urticaria with persistent weals, 705
Congenital pigmented naevi, epidemiology, 307
Connective tissue disease, 713
Contact allergy to clioquinol and neomycin (Summary), 100
Contact dermatitis due to an epoxy acrylate, 697
Contact dermatitis modified by PUVA, 165
Contact dermatitis modified by UV-B, 161
Corticosteroid atrophy and the Duhring chamber, 57
Corticosteroids and halogenation, a comparison, 179
Cowden's disease (Case report), 107
Culture of hair follicle cells, 1
Cutaneous body image, 611
Cutaneous manifestations of paraproteinaemia Part II, 209
Cutaneous T cell lymphomas, diagnosis, 257
Cyclic GMP-phosphodiesterase in psoriasis, 301

Dantrolene causing acne, 465
Dapsone in lupus erythematosus, 53
Dapsone treated dermatitis herpetiformis and hypoalbuminaemia, 201
Dapsone unsuccessful as treatment of alopecia areata, 597
Darier's disease treated with Tigason, 675
Demethylchlortetracycline and griseofulvin for mycosis fungoides, 477
Dermal microvasculature, 117
Dermatitis allergic contact due to an epoxy acrylate, 697
Dermatitis, contact, modified by PUVA, 165
Dermatitis, contact, modified by UV-B, 161
Dermatitis herpetiformis and HLA-DR3, 321
Dermatitis herpetiformis treated with dapsone and hypoalbuminaemia, 201
Dermatitis herpetiformis with cerebellar ataxia and sensory neuropathy, 593
Dermatological non-disease, 611
Dermographism, cimetidine and chlorpheniramine, 185
Discoid lupus erythematosus-like lesions in X-linked chronic granulomatous disease, 495
Disulphide and sulphhydryl stainings of subepidermal hyaline bodies, 437
Duhring chamber to assay corticosteroid atrophy, 57

Editorial, 729
Electronmicroscopy. Cutaneous ultrastructural diagnosis of ceroid-lipofuscinosis, 581
Electron microscopy. Human dermal microvasculature, 117
Electron microscopy. Ultrastructure of white spots in tuberous sclerosis (Summary), 100
Encephalocraniocutaneous lipomatosis, 89
Enzyme study in human dermal microvasculature, 521
Enzymes. Acid α-naphthyl acetate esterase as a marker for T lymphocytes, 31
Epidemiology of congenital pigmented naevi I, 307
Epidermal cell glycoconjugate turnover, 659
Epidermal cell kinetics in pityriasis rubra pilaris, 249
Epidermis and human ageing, 627
Epidermis. Vincristine and colcemid used to measure proliferative rates, 243
Epidermolysis bullosa acquisita (Title), 102
Epoxy acrylate causing allergic contact dermatitis, 697
Erythema multiforme caused by mianserin, 481
Erythema multiforme treated with zinc sulphate, 191
Erythromycin and tetracycline inhibiting lipase production in *Propionibacterium acnes*, 453
Erythropoietic protoporphyria and liver disease, 7
Eye protection in PUVA, 295

Factitious urticaria: red dermographism, 285
Factitious urticaria treated by cimetidine and chlorpheniramine, 185
Flare and weal response to histamine, 27
Flow cytometry for cell kinetics 2, 271
Follicular psoriasis, 153

Genodermatoses. Epidemiology of congenital pigmented naevi II. Risk factors, 421
Genodermatoses. Pityriasis rubra pilaris and retinol-binding protein, 253
Genodermatoses. Richner-Hanhart syndrome, 469
Genodermatoses. Tuberous sclerosis (Summary), 99
GERL tyrosinase-rich in melanin synthesis, 169
D-(^{3}H) glucosamine in normal and psoriatic epidermis, 429
Glycoconjugate turnover in epidermis, 649
Glycoconjugates and psoriatic keratinocytes, 635
GM_1 gangliosidosis type I and mongolian spots, 195

Granulomatous disease, X-linked chronic, and discoid lupus erythematosus-like lesions, 495
Griseofulvin and demethylchlortetracycline in mycosis fungoides, 477
Grotan BK patch test concentrations (Correspondence), 607

Hair follicle cell culture, 1
Halogenation and topical corticosteroids, a comparison, 179
Hepatitis B and urticaria, 383
Herpes gestationis and anti-HLA immunization, 553
Herpes simplex trated with zinc sulphate solution, 191
Herpes Zoster (Correspondence), 351
Herpetiform pemphigus (Case report), 102
Histamine and weal and flare response, 27
Histamine receptors in human skin, 601
HLA antigens and scabies, 317
HLA-DR3 in dermatitis herpetiformis, 321
Huber's stain, a modification, 721
Hyaline subepidermal bodies stained with sulphhydryl and disulphide, 437
Hypertrichosis languinosa congenital (Case report), 108
Hypoalbuminaemia in dapsone treated dermatitis herpetiformis, 201

Immune response to adjuvants in penicillin preparations (Summary), 97
Immunofluorescent patterns of healthy skin (Title), 101
Immunoglobulins in lichen planus, 207
Immunology. Herpes gestationis and anti-HLA immunization, 553
Immunology. The modulating actions of oral retinoid, 37
Immunomodulatory activity of oral retinoid, 37
Ingram method for psoriasis followed by low-dose PUVA, 681
International Committee of Dermatology. Report of Meeting, 227

Jessner's lymphocytic infiltrate, lupus erythematosus, T lymphocytes and mononuclear cells, 141
Juvenile plantar dermatosis in an adult, 599

Keratinization disorders and Tigason, 667
Keratinocytes and lectin binding, 641
Keratinocytes in psoriasis and uptake of sugars, 635
Ketotifen and terbutaline in urticaria, 205
Kinetics of metaphase arrest in psoriasis, 231

Lectin and epidermal keratinocytes, 641
Lentiginous naevus (Case report), 104
Lichen planus, serum immunoglobulins, 207
Lichen sclerosus et atrophicus and auto-immune disorders, 563
Lichen sclerosus et atrophicus and HLA-B40, 557
Lichen urticatus in an Indonesian (Summary), 98
Light sensitivity in malignant melanoma, 289
Lipase inhibition by tetracycline and erythromycin, 453
Lipid absorption of ultraviolet radiation, 549
Lipomatosis encephalocraniocutaneous, 89
Liver disease in erythropoietic protoporphyria, 7
Lupus erythematosus and bullous pemphigoid, 71
Lupus erythematosus and Jessner's lymphocytic infiltrate, T lymphocytes and mononuclear cells, 141
Lupus erythematosus, discoid, a simple sign (Correspondence), 350
Lupus erythematosus treated with dapsone, 53
Lymphadenopathy, angioimmunoblastic, skin lesions in, 131
Lymphangiofibrosis thrombotica occlusive, 687
Lymphocyte function and PUVA, 405
Lymphoma, non-Hodgkin's, of skin, 333
Lymphoma with lymphomatoid papulosis, 77
Lymphomatoid papulosis and lymphoma, 77

Malignant cutaneous lesions, loss of cell surface β_2 microglobulin, 507
Melanin synthesis and tyrosinase-rich GERL, 169
Melanoma malignant and light sensitivity, 289
Melanoma management, changing concepts, 341
8-Methoxypsoralen induced sunburn cells, 541
8-Methoxypsoralen plasma concentrations in photochemotherapy of psoriasis, 47
Mianserin causing erythema multiforme, 481
β_2 Microblobulin loss in malignant and premalignant lesions, 507
β_2 Microglobulin present in premalignant skin disorders, 515
Microvasculature, human dermal, II. Enzyme study, 521
Microvasculature of the skin, 117
Molluscum contagiosum (Title), 101
Mongolian spots in GM_1 gangliosidosis type I, 195
Mycosis fungoides treated with demethylchlortetracycline and griseofulvin, 477
Mycotic infections (Title), 102

Naevi, congenital pigmented, epidemiology II, 421
Naevi, pigmented, epidemiology, 307
Neomycin and clioquinol allergy (Summary), 100
News and Notices, 115, 229, 359, 493, 610, 730
Nictoine: an hapten (Correspondence), 349
Non-Hodgkin's lymphoma of skin, 333

Obituaries
  Dr Harold Blum, 358
  Dr D.M.Pilsbury, 358
Occupational argyria, 19

Palmo-plantar dermatoses treated with PUVA (Title), 101
Paraproteinaemia, cutaneous manifestations Part II, 209
Pemphigoid and pemphigus vulgaris, factors in prognosis, 415
Pemphigoid, bullous, and systemic lupus erythematosus, 71
Pemphigus herpetiform (Case report), 102
Pemphigus vegetans and cerebriform tongue, 587

Pemphigus vulgaris and pemphigoid and prognosis, 415
Penicillin allergy and chronic urticaria (Summary), 98
Penicillin allergy, repeated, patch testing, 157
Penicillin hypersensitivity (Title), 102
Penicillin preparations, immune response to adjuvants (Summary), 97
Penis, sclerosing lymphangiitis, 687
Phenolic compounds and tyrosinase inhibition, 659
Photochemotherapy and eye protection, 295
Photochemotherapy of psoriasis and plasma 8-methoxypsoralen concentrations, 47
Pigmented naevi, congenital, epidemiology of II, 421
Pigmented naevi, epidemiology, 307
Pityriasis rubra pilaris and retinol-binding protein, 253
Pityriasis rubra pilaris: epidermal cell kinetics, 249
Plantar dermatosis in an adult, 599
Porphyria cutanea tarda and haemodialysis, 575
Porphyria cutanea tarda and haemodialysis, 579
Premalignant and malignant cutaneous lesions, loss of cell surface β_2 microglobulin, 507
Premalignant skin disorders and β_2 microglobulin, 515
Primula obconica used in alopecia areata, 339
Proliferative rates of epidermis using vincristine and colcemid, 243
Propionibacterium acnes lipase production inhibited by tetracycline and erythromycin, 453
17α Propyltestosterone and human sebum excretion, 685
Protease inhibitor autoantibodies, 621
Protoporphyria, erythropoietic, and liver disease, 7
Psoralen/UV-A modifying contact dermatitis, 165
Psoriasis and plasma 8-methoxypsoralen concentrations with photochemotherapy, 47
Psoriasis, aryl hydrocarbon hydroxylase activity in (Correspondence), 352
Psoriasis cell kinetic data by flow cytometry, 271
Psoriasis follicular, 153
Psoriasis, metabolism of cyclic GMP, 301
Psoriasis. The effect of oral retinoid on dermal components, 37
Psoriasis treated by Ingram method maintained by low-dose PUVA, 681
Psoriasis treated with aromatic retinoid, PUVA and retinoid and PUVA alone compared, 325
Psoriatic epidermis and incorporation of D-(^{3}H)-glucosamine, 429
Psoriatic epidermis, kinetics of metaphase arrest, 231
Psoriatic keratinocytes, uptake of sugars, 635
PUVA and eye protection, 295
PUVA and lymphocyte function, 405
PUVA low-dose maintenance in psoriasis, 681
PUVA photosensitization of *Trichophyton*, 397
PUVA used with aromatic retinoid in psoriasis, 325
Pyoderma gangrenosum and haematological malignancies (Correspondence), 349
Pyoderma gangrenosum, idiopathic, and therapy with azothioprine and corticosteroid, 567

Recall antigens skin reactions (Summary), 101
Recurrent urticaria, 369
Retinoid orally having an immunomodulatory activity, 37
Retinol-binding protein in pityriasis rubra pilaris, 253
Richner-Hanhart syndrome (tyrosinaemia-II), 469
Ro 10-9359 in Darier's disease, 675
Ro 12-9359 in disorders of keratinization, 667

Scabies, HLA antigens in, 317
Sclerosing lymphangiitis of the penis, 687
Seborrhoeic keratosis, acid glycosidases in, 147
Sebum excretion inhibited by topical 17α propyltestosterone, 685
Sensory neuropathy and cerebellar ataxia in dermatitis herpetiformis, 593
Skin disease and albumin, 389
Skin, early changes during carcinogenesis, 485
Stevens-Johnson syndrome followed by pigmented naevi (Case report), 106
Sulphhydryl and disulphide stainings of subepidermal hyaline bodies, 437
Sunburn cells and 8-methoxypsoralen, 541

T cell lymphomas, diagnosis, 257
Terbutaline and ketotifen in urticaria, 205
Tetracycline and erythromycin inhibiting lipase production in *Propionibacterium acnes*, 453
Tigason in Darier's disease, 675
Tigason in disorders of keratinization, 667
Tigason in psoriasis, a comparison, 325
T lymphocytes, acid α-naphthyl acetate esterase as a marker, 31
T lymphocytes and mononuclear phagocytes in lupus erythematosus and Jessner's lymphocytic infiltrate, 141
Tobacco dermatitis (Correspondence), 349
Treatment. A comparison between halogenation and topical corticosteroids, 179
  Alopecia areata treated with *Primula obconica*, 339
  AMP for herpes zoster (Correspondence), 351
  Colchicine for Behçet's disease, 67
  Dapsone in the treatment of lupus erythematosus, 53
  Dapsone unsuccessful in alopecia areata, 597
  Demethylchlortetracycline and griseofulvin for mycosis fungoides, 477
  Effects of Tigason in severe disorders of keratinization, 667
  Factitious urticaria treated by cimetidine and chlorpheniramine, 185
  Failure of azothioprine and corticosteroid therapy in idiopathic pyoderma gangrenosum, 567
  Ketotifen and terbutaline in urticaria, 205
  Low-dose PUVA maintenance in psoriasis following Ingram therapy, 681
  of psoriasis. A comparison of aromatic retinoid, PUVA with retinoid and PUVA alone, 325
  Oral zinc sulphate in alopecia areata, 483
  Serum levels of trimethypsoralen, 443
  Tigason in Darier's disease, 675
  Topical zinc sulphate for herpes simplex and erythema multiforme, 191
Trichophyton photosensitized by PUVA, 397
Trimethylpsoralen serum levels after oral administration, 443
Tuberous sclerosis (Summary), 99

Tuberous sclerosis. Ultrastructure of white spots (Summary), 100
Tyrosinaemia-II, Richner-Hanhart syndrome, 469
Tyrosinase inhibitors and phenolic compounds, 659
Tyrosinase inhibitors, biochemical characterization, 531

Ultraviolet radiation absorption by skin surface lipid, 549
Ultravillet radiation and contact dermatitis I. UV-B radiation, 161
Ultraviolet radiation and contact dermatitis II. Psoralen/UV-A, 165
Urticaria and acute hepatitis B, 383
Urticaria and penicillin allergy (Summary), 98
Urticaria, cold, with persistent weals, 705
Urticaria, factitious, cimetidine and chlorpheniramine, 185
Urticaria factitious, red dermographism, 285
Urticaria, localized cold reflex, 83
Urticaria recurrent, 369
Urticaria treated with ketotifen and trebutaline, 205
UV-B radiation modifying allergic contact dermatitis, 161
UV fluorescent lamps, a cautionary note (Correspondence), 607

Vincristine and colcemid to measure proliferative rates in epidermis, 243

Weal and flare response to histamine, 27

Xanthomatosis cutis with rare manifestations (Case report), 110

Zinc deficiency and acrodermatitis enteropathica, 459
Zince sulphate in alopecia areata, 483
Zinc sulphate solution for recurrent herpes simplex and post-herpetic erythema multiforme, 191
Zosteriform lentiginous naevus (Case report), 104

AUTHORS

Allenby, C.F. and Sparkes, C.G. Halogenation and topical corticosteroids: a comparison between the 17-butyrate esters of hydrocortisone and clobetasone in ointment bases, 179
Altomare, G. *see* Finzi, A.F.

Babb, E.J. *see* Lichtenstein, J.R.
Basset, A. *see* Eberts, E.
Bauer, F.W., Crombag, N.H.C.M.N., Boezeman, J.B.M. and De Grood, R.M. Flow cytometry as a tool for the study of cell kinetics in skin 2. Cell kinetic data in psoriasis, 271
Beadle, P.C. and Burton, J.L. Absorption of ultraviolet radiation by skin surface lipid, 549
Behrenbeck, E.-M. *see* Frosch, P.J.
Beitner, H., Ringborg, U., Wennersten, G. and Lagerlöf, B. Further evidence for increased light sensitivity in patients with malignant melanoma, 289
Bergamaschini, L. *see* Finzi, A.F.
Bergers, M. *see* Roelfzema, H.
Bernengo, M.G., Levi, L. and Zina, G. Skin lesions in angioimmunoblastic lymphadenopathy: histological and immunological studies, 131
Berretty, P.J.M. *see* Neumann, H.A.M.
Bibby, M.C. The specificity of early changes in the skin during carcinogenesis, 485
Black, C. Mixed connective tissue disease, 713
Bleehen, S.S., Gould, D.J., Harrington, C.I., Durrant, T.E., Slater, D.N. and Underwood, J.C.E. Occupational argyria; light and electron microscopic studies and X-ray microanalysis, 19
Bloch, K.J. *see* Morison, W.L.
Bloemèndal, H. *see* Weterings, P.J.J.M.
Blom, I., Jameson, S., Krook, F., Larsson-Stymne, B. and Wranne, L. Zinc deficiency with transitory acrodermatitis enteropathica in a boy of low birth weight, 459
Boezeman, J.B.M. *see* Bauer, F.W.
Boonk, W.J. and Van Ketel, W.G. Chronic urticaria and penicillin allergy (Summary), 98
Boonk, W.J. *see* Bruynzeel, D.P.
Boorsma, D.M., Nieboer, C., Woerdeman, M.J. and Kalsbeek, G.L. Epidermolysis bullosa aquisita: an (immune) electronmicroscopic and immunohistochemical study (Title), 102
Brandrup, F., Koch, C., Petri, M., Schiødt, M. and Johansen, K.S. Discoid lupus erythematosus-like lesions and stomatitis in female carriers of X-linked chronic granulomatous disease, 495
Breathnach, S.M., Wells, G.C. and Valdimarsson, H. Idiopathic pyoderma gangrenosum and impaired lymphocyte function: failure of azothioprine and corticosteroid therapy, 567
Brody, I. Topical treatment of recurrent herpes simplex and post-herpetic erythema multiforme with low concentrations of zinc sulphate solution, 191
Bruynzeel, D.P. and Boonk, W.J. Treatment of chronic palmo-plantar dermatoses with PUVA (Title), 101
Bruynzeel, D.P. and Van Ketel, W.G. Repeated patch testing in penicillin allergy, 157
Burge, S.M., Wilkinson, J.D., Millar, A.J. and Ryan, T.J. The efficacy of an aromatic retinoid, Tigason (Ro 10-9359), in the treatment of Darier's disease, 675
Burton, J.L. *see* Beadle, P.C.

Camplejohn, R.S., Gelfant, S. and Chalker, D. An attempt to use vincristine and colcemid to measure proliferative rates in normal human epidermis *in vivo*, 243
Cancarini, G.C. *see* Topi, G.C.
Cantieri, J.S., Graff, G. and Goldberg, N.D. Cyclic GMP metabolism in psoriasis: increased activity of soluble epidermal cyclic GMP phosphodiesterase and its modulation by calcium, 301
Caro, W.A. *see* Weissbluth, M.
Castilla, E.E., Duthra, M.D.G. and Orioli-Parreiras, I.M. Epidemiology of congenital pigmented naevi: I. Incidence rates and relative frequencies, 307
Castilla, E.E., Dutra, M.D.G. and Orioli-Parreiras, I.M. Epidemiology of congenital pigmented naevi: II. Risk factors, 421
Chalker, D. *see* Camplejohn, R.S.
Chapman, P.H. *see* Shuster, S.
Chu, A.C., Morgan, E.W. and Macdonald, D.M. Evaluation of acid α-naphthyl acetate esterase activity as a marker of T lymphocytes in benign and neoplastic cutaneous infiltrates, 31
Cirkel, P.K.S. *see* Starink, Th.M.
Clemmensen, O.J. *see* Worm, A.-M.
Connors, T.J. *see* Czarnecki, D.B.
Cook, L.J. and Shuster, S. The effect of placebo and 'aggro' on the weal and flare response to histamine, 27
Cormane, R.H. *see* Neumann, H.A.M.
Cormane, R.H. *see* Stolk, L.
Cornelisse, C.J. *see* Van der Loo, E.M.
Cotterill, J.A. Dermatological non-disease: a common and potentially fatal disturbance of cutaneous body image, 611
Cowan, R.E. and Wright, J.T. Dapsone and severe hypoalbuminaemia in dermatitis herpetiformis, 201
Cowen, P.S. *see* Czarnecki, D.B.
Crombag, N.H.C.M.N. *see* Bauer, F.W.
Czarnecki, D.B., Cowen, P.S. and Connors, T.J. Juvenile plantar dermatosis in an adult, 599
Czarnetzki, B.M., Frosch, P.J. and Sprekeler, R. Localized cold reflex urticaria, 83

Dahl, M.G.C. Patch test concentrations of grotan BK (Correspondence), 607
D'Alessandro, G.L. *see* Topi, G.C.
Daneshmend, T.K. and Gardner-Thorpe, C. Cerebellar ataxia and sensory neuropathy associated with dermatitis herpetiformis, 593

Danno, K. and Horio, T. Sulphhydryl and disulphide stainings of subepidermal hyaline bodies, 437

Davey, J.B., Diffey, B.L. and Miller, J.A. Eye protection in psoralen photochemotherapy, 295

Davies, H.W. and Trotter, M.D. Synthesis and turnover of membrane glycoconjugates in monolayer culture of pig and human epidermal cells, 649

Davies, M.G. and Greaves, M.W. The current status of histamine receptors in human skin: therapeutic implications, 601

Dawber, R. *see* Ralfs, I.

Dawber, R.P.R. *see* Ralfs, I.G.

De Bersaques, J. Acid glycosidases in basal cell epithelioma and in seborrhoeic keratosis, 147

De Bersaques, J. Arylsulphatases in tumours (Correspondence), 221

De Constanza, F. *see* Topi, G.C.

De Grood, R.M. *see* Bauer, F.W.

De Groot, W.P., Woerdeman, M.J., Witkiewicz, I.M., Fleury, P., Delleman, J.W. and Verbeeten, B. Tuberous sclerosis. An investigation into the ratio of sporadic versus familial cases (Summary), 99

De Groot, W.P. *see* Witkiewicz, I.M.

De Haan, P. and Kalsbeek, G.L. Immune response due to adjuvants in penicillin preparations (Summary), 97

De Haan, P., Kalsbeek, G.L. and Van Ketel, W.G. Correlation between *in vitro* and *in vivo* tests in penicillin hypersensitivity (Title), 102

Delleman, J.W. *see* De Groot, W.P.

Diffey, B.L. A cautionary note on u.v. fluorescent lamps (Correspondence), 607

Diffey, B.L. *see* Davey, J.B.

Dirksen, H.J. *see* Van der Horst, J.C.

Dolman, W. *see* Rhodes, E.L.

Dorp, B.K.-V. *see* Starink, Th.M.

Duffill, M. *see* Ralfs, I.

Dunsmore, I.R. *see* Harrington, C.I.

Durrant, T.E. *see* Bleehen, S.S.

Dutra, M.D.G. *see* Castilla, E.E.

Ead, R.D. Oral zinc sulphate in alopecia areata—a double blind trial, 483

Eady, R.A.J. *see* Higgins, J.C.

Eberst, B. *see* Eberst, E.

Eberst, E., Tongio, M.M., Eberst, B., Mayer, S. and Basset, A. Herpes gestationis and anti-HLA immunization, 553

Eftekhari, N. *see* Kaur, S.

Esterly, N.B. *see* Weissbluth, M.

Ewen, S.W.B. *see* Stankler, L.

Falk, E.S. and Thorsby, E. HLA antigens in patients with scabies, 317

Felsher, B.F. *see* Lichtenstein, J.R.

Feuerman, E.J. Pyoderma gangrenosum and haematological malignancies (Correspondence), 349

Finlay, A.Y. *see* Marks, R.

Finzi, A.F., Altomare, G., Bergamascini, L. and Tucci, A. Pityriasis rubra pilaris and retinol-binding protein, 253

Fleury, P. *see* De Groot, W.P.

Folkers, E., Schouten, J.A. and Thöne, A.W. Xanthomatosis cutis in a patient with hyperlipoproteinaemia, Frederickson type IIB, with unusual localization and cystic lesions (Case report), 110

Frain-Bell, W. *see* Stevenson, I.H.

Friedmann, P.S. Unsuccessful treatment of alopecia areata with dapsone, 597

Frosch, K. *see* Frosch, P.J.

Frosch, P.J., Behrenbeck, E.-M., Frosch, K. and Macher, E. The Duhring chamber assay for corticosteroid atrophy, 57

Frosch, P.J. *see* Czarnetzki, B.M.

Gardner-Thorpe, C. *see* Daneshmend, T.K.

Gelfant, S. *see* Camplejohn, R.S.

Gelsthorpe, K. *see* Harrington, C.I.

Germain, D. *see* MacDonald, D.M.

Goerz, G. *see* Ruzicka, T.

Goldberg, N.D. *see* Cantieri, J.S.

Gommans, J.M. and Van den Hurk, J.J.M.A. Lectin binding studies on suspensions of isolated epidermal keratinocytes, 641

Gommans, J.M. *see* Mier, P.D.

Gommans, J.M. *see* Roelfzema, H.

Gould, D.J. *see* Bleehen, S.S.

Graff, G. *see* Cantieri, J.S.

Greaves, M.W. *see* Davies, M.G.

Greaves, M. *see* Kaur, S.

Griso, D. *see* Topi, G.C.

Hamada, T. *see* Ishii, M.

Harrington, C.I. and Dunsmore, I.R. An investigation into the incidence of auto-immune disorders in patients with lichen sclerosus and atrophicus, 563

Harrington, C.I. and Gelsthorpe, K. The association between lichen sclerosus et atrophicus and HLA-B40, 557

Harrington, C.I. *see* Bleehen, S.S.

Herken, R. and Schultz-Ehrenburg, U. Autoradiographic investigations on the cell kinetics of epidermis and periderm of limb buds from mouse embryos *in vitro*, 277

Higami, S. *see* Ishii, M.

Higgins, J.C. and Eady, R.A.J. Human dermal microvasculature: I. Its segmental differentiation. Light and electron microscopic study, 117

Higgins, J.C. and Eady, R.A.J. Human dermal microvasculature: II. Enzyme histochemical and cytochemical study, 521

Holt, P.J.A. *see* Marks, R.

Holubar, K. *see* Pehamberger, H.

Hopsu-Havu, V.K., Kalimo, U. and Jarvinen, M. Autoantibodies to an epidermal protease inhibitor in skin diseases, 621

Horio, T. and Watanabe, S. Photosensitization of *Trichophyton* with 8-methoxypsoralen to UV-A light radiation, 397

Horio, T. *see* Danno, K.

Horio, T. *see* Miyachi, Y.

Husz, S. *see* Szabó, E.

Imokawa, G. and Mishima, Y. Biochemical characterization of tyrosinase inhibitors using tyrosinase binding affinity chromatography, 531

Imokawa, G. and Mishima, Y. Isolation and biochemical characterization of tyrosinase-rich GERL and coated vesicle in melanin synthesizing cells, 169

Ishii, M., Takahashi, K., Hamada, T., Tanaka, A. and Higami, S. Cutaneous ultrastructural diagnosis of ceroid-lipofuscinosis, 581

Jameson, S. *see* Blom, I.

Jarvinen, M. *see* Hopsu-Havu, V.K.

Jayakumar, S. *see* Premalatha, S.

Johansen, K.S. *see* Brandrup, F.

Johnson, B.E. *see* Stevenson, I.H.

Juhlin, L. Cold urticaria with persistent weals, 705

Juhlin, L. Recurrent urticaria: clinical investigation of 330 patients, 369

Juvakoski, T. *see* Lauharanta, J.

Kalimo, U. *see* Hopsu-Havu, V.K.

Kalsbeek, G.L. *see* Boorsma, D.M.

Kalsbeek, G.L. *see* De Haan, P.

Kataria, M. *see* Pembroke, A.C.

Kaur, S., Greaves, M. and Eftekhari, N. Factitious urticaria(dermographism): treatment by cimetidine and chlorpheniramine in a randomized double-blind study, 185

Kenicer, K.J.A. *see* Stevenson, I.H.

Kennedy, C. *see* Rhodes, E.L.

Kernekamp, A.S.-V.W. *see* Van Ketel, W.G.

Kersey, P.J. *see* Shuster, S.

King, I.A., Tabiowo, A. and Paul, C.J. Incorporation of D-(^{3}H)glucosamine into normal and psoriatic epidermal glycoconjugates, 429

Koch, C. *see* Brandrup, F.

Konttinen, Y.T., Reitama, S., Ranki, A. and Segerberg-Konttinen, M. T lymphocytes and mononuclear phagocytes in the skin infiltrate of systemic and discoid lupus erythematosus and Jessner's lymphocytic infiltrate, 141

Korthals, H.R., Van Vinninghe, A.-L. and Neumann, H.A.M. The presence of B_2 microglobulin on the membrane of the keratinocyte in premalignant skin disorders, 515

Kovács, L. *see* Szabó, E.

Krook, F. *see* Blom, I.

Krugler, J.I. *see* Morison, W.L.

Lagerlöf, B. *see* Beitner, H.

Landwehr, A.J. Congenital hypertrichosis lanuginosa (Case report), 108

Larsson-Stymne, B. *see* Blom, I.

Lassus, A. *see* Lauharanta, J.

Lauharanta, J., Juvakoski, T. and Lassus, A. A clinical evaluation of the effects of an aromatic retinoid (Tigason), combination of retinoid and PUVA and PUVA alone in severe psoriasis, 325

Levi, L. *see* Bernengo, M.G.

Leyden, J.J. *see* Webster, G.F.

Lichtenstein, J.R., Babb, E.J. and Felsher, B.F. Porphyria cutanea tarda (PCT) in a patient with chronic renal failure on haemodialysis, 575

Lyons, F. and Shuster, S. Effect of topical $17\,\alpha$ Propyltestosterone on sebum excretion in man, 685

MacDonald, D.M., Germain, D. and Perrot, H. The histopathology and ultrastructure of liver disease in erythropoietic protoporphyria, 7

MacDonald, D.M. *see* Chu, A.C.

McGinley, K.J. *see* Webster, G.F.

Macher, E. *see* Frosch, P.J.

Mackie, R.M. *see* Turbitt, M.L.

Magnus, I.A. *see* Young, A.R.

Mahood, J.M. Serum immunoglobulins in lichen planus, 207

Mali, J.W.H. *see* Van de Kerkhof, P.C.M.

Mandell, F. *see* Sanchez, N.P.

Marks, R. Measurement of biological ageing in human epidermis, 627

Marks, R., Finlay, A.Y. and Holt, P.J.A. Severe disorders of Keratinization: effects of treatment with Tigason (Ro 10-9359), 667

Marsch, W.Ch. and Stuttgen, G. Sclerosing lymphangiitis of the penis: a lymphangiofibrosis thrombotica occlusiva, 687

Marshall, T.L. *see* Messenger, A.G.

Mayer, S. *see* Eberts, E.

Mayr, W.R. *see* Pehamberger, H.

Meijer, C.J.L.M. *see* Van der Loo, E.M.

Messenger, A.G., Marshall, T.L. and Summerly, R. A case of lymphomatoid papulosis and systemic lymphoma, 77

Meyer, C.J.L.M. *see* Willemze, R.

Mier, P.D., Gommans, J.M. and Roelfzema, H. Aryl hydrocarbon hydroxylase activity in psoriasis (Correspondence), 353

Mier, P.D. *see* Orfanos, C.E.

Mier, P.D. *see* Roelfzema, H.

Mihm, M.C. *see* Sanchez, N.P.

Millar, A.J. *see* Burge, S.M.

Miller, J.A. *see* Davey, J.B.

Mishima, Y. *see* Imokawa, G.

Miyachi, Y., Taniguchi, S., Ozaki, M. and Horio, T. Colchicine in the treatment of the cutaneous manifestations of Behçet's disease, 67

Morgan, E.W. *see* Chu, A.C.

Morison, W.L., Parrish, J.A., Bloch, K.J. and Krugler, J.I. *In vivo* effects of PUVA and lymphocyte function, 405

Morison, W.L., Parrish, J.A., Woehler, M.E. and Bloch, K.J. The influence of ultraviolet radiation on allergic contact dermatitis in the guinea-pig. I. UV-B radiation, 161

Morison, W.L., Parrish, J.A., Woehler, M.E. and Bloch, K.J. The influence of ultraviolet radiation on allergic contact dermatitis in the guinea-pig. II. Psoralen/UV-A radiation, 165

Nazzaro-Porro, M. *see* Passi, S.

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Brief Communications

An unusual adverse reaction to Ro 10-9359

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We have administered retinoid (Ro 10-9359, Tigason) alone in seventeen selected cases of dermatoses (psoriasis vulgaris—4, pustular psoriasis—2, psoriatic arthritis—1, psoriatic nails—1, palmoplantar keratoderma—1, vitiligo—5, severe acne—3), and we have observed an adverse reaction which is not referred to in the literature, as far as we know. The patients were followed up for a period of 10 months. The untoward effect of the drug occurred in a female patient, aged 33 years, suffering from severe psoriasis vulgaris since 1970 and psoriatic arthritis of the hands and especially in the proximal interphalangeal joints of the right index and middle fingers since 1979. The drug was administered in a dose of 1 mg/kg (70 mg per day) for 20 days three times in a period of 6 months.

Fourteen days after the initiation of treatment, the patient developed generalized oedema. The oedema started in the face and extended rapidly to the upper and lower extremities, the thorax and the abdomen. The colour of the skin remained unchanged. The swelling caused discomfort to such a degree that she could not bear her belt and her shoes did not fit her any more. Moderate pitting was obvious in the feet. There was no sign of inflammation or pain. Her face simulated that observed after prolonged treatment with steroids. Laboratory investigations such as blood count, sedimentation rate, serum bilirubin, SGOT, SGPT, alkaline phosphatase, K, Na, serum immunoglobulins, serum proteins, urinalysis and liver function tests were all normal.

The drug was discontinued and the oedema entirely disappeared on the seventh day after cessation. The drug was given again and the same phenomenon was observed twice during the administration of the drug, each time at the end of the second week.

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Neering, H. Retarded (5–7 day) skin reactions to recall antigens (Summary), 101

Neering, H. and Van der Horst, J.C. Immunologic aspects of mycotic infections (Title), 102

Nethercott, J.R. Allergic contact dermatitis due to an epoxy acrylate, 697

Neumann, H.A.M., Berretty, P.J.M., Reinders Folmer, S.C.C. and Cormane, R.H. Hepatitis B surface antigen deposition in the blood vessel walls of urticarial lesions in acute hepatitis B, 383

Neumann, H.A.M. *see* Korthals, H.R.

Nieboer, C. Patterns of immunofluorescence of the skin in healthy individuals (Title), 101

Nieboer, C. *see* Boorsma, D.M.

Orfanos, C.E., Roelfzema, H. and Mier, P.D. Arylsulphatases in tumours (Correspondence), 222

Orfanos, C.E. *see* Tsambaos, D.

Orioli-Parreiras, I.M. *see* Castilla, E.E.

Ozaki, M. *see* Miyachi, Y.

Parrish, J.A. *see* Morison, W.L.

Parving, H.-H. *see* Worm, A.-M.

Passi, S. and Nazzaro-Porro, M. Molecular basis of substrate and inhibitory specificity of tyrosinase: phenolic compounds, 659

Paul, C.J. *see* King, I.A.

Pehamberger, H., Holubar, K. and Mayr, W.R. HLA-DR3 in dermatitis herpetiformis, 321

Pembroke, A.C., Saxena, S.R., Kataria, M. and Zilkha, J.D. Acne induced by dantrolene, 465

Perrot, H. *see* MacDonald, D.M.

Petri, M. *see* Brandrup, F.

Premalatha, S., Jayakumar, S., Yesudian, P. and Thambiah, A.S. Cerebriform tongue—a clinical sign in pemphigus vegetans, 587

Quraishy, E. Erythema multiforme during treatment with mianserin—a case report, 481

Ralfs, I., Dawber, R., Ryan, T., Duffill, M. and Wright, N.A. The kinetics of metaphase arrest in human psoriatic epidermis: an examination of optimal experimental conditions for determining the birth rate, 231

Ralfs, I.G., Dawber, R.P.R., Ryan, T.J. and Wright, N.A. Pityriasis rubra pilaris: epidermal cell kinetics, 249

Rampen, F. Changing concepts in melananoma management, 341

Ranki, A. *see* Konttinen, Y.T.

Ravelli, M. *see* Topi, G.C.

Rawlins, M.D. *see* Shuster, S.

Rehák, A., Selim, M.M. and Yadav, G. Richner-Hanhart syndrome (tyrosinaemia-II) (report of four cases without ocular involvement), 469

Reinders Folmer, S.C.C. *see* Neumann, H.A.M.

Reitamo, S. *see* Konttinen, Y.T.

Rhodes, A.R. *see* Sanchez, N.P.

Rhodes, E.L., Dolman, W., Kennedy, C. and Taylor, R.R. Alopecia areata regrowth induced by *Primula obconica*, 339

Ringborg, U. *see* Beitner, H.

Roelfzema, H., Bergers, M., Van Erp, P.E.J., Gommans, J.M. and Mier, P.D. Studies on the plasma membrane of normal and psoriatic keratinocytes 3. Uptake of labelled sugars and their incorporation in glycoconjugates, 635

Roelfzema, H. *see* Mier, P.D.

Roelfzema, H. *see* Orfanos, C.E.

Rossing, N. *see* Worm, A.-M.

Russell Jones, R. Cutaneous manifestations of paraproteinaemia. Part II, 209

Ruzicka, T. and Goerz, G. Dapsone in the treatment of lupus erythematosus, 53

Ryan, T. *see* Ralfs, I.

Ryan, T.J. *see* Burge, S.M.

Ryan, T.J. *see* Ralfs, I.G.

Saihan, E.M. Ketotifen and terbutaline in urticaria 205

Sanchez, N.P., Rhodes, A.R., Mandell, F. and Mihm, M. Encephalocraniocutaneous lipomatosis: a new neurocutaneous syndrome, 89

Savin, J.A. Some factors affecting prognosis in pemphigus vulgaris and pemphigoid, 415

Saxena, S.R. *see* Pembroke, A.C.

Scheffer, E. *see* Van der Loo, E.M.

Schiødt, M. *see* Brandrup, F.

Schouten, J.A. *see* Folkers, E.

Schultz-Ehrenburg, U. *see* Herken, R.

Segerberg-Konttinen, M. *see* Konttinen, Y.T.

Selim, M.M. *see* Rehák, A.

Shelley, W.B. Demethylchlortetracycline and griseofulvin as examples of specific treatment for mycosis fungoides, 477

Shuster, S. A simple sign of lupus erythematosus (Correspondence), 350

Shuster, S., Rawlins, M.D., Kersey, P.J. and Chapman, P.H. Aryl hydrocarbon hydroxylase activity in psoriasis (Correspondence), 352

Shuster, S. *see* Cool, L.J.

Shuster, S. *see* Lyons, F.

Siddiqui, A.H. *see* Stolk, L.

Sklar, S.H. and Wigand, J.S. Herpes zoster (Correspondence), 351

Slater, D.N. *see* Bleehen, S.S.

Sparkes, C.G. *see* Allenby, C.F.

Sprekeler, R. *see* Czarnetzki, B.M.

Stankler, L. and Ewen, S.W.B. Follicular psoriasis, 153

Starink, Th.M., Cirkel, P.K.S. and Dorp, B.K.-V. Cowden's disease (Case report), 107

Sterboul, J. *see* Sudan, B.J.J.

Stevenson, I.H., Kenicer, K.J.A., Johnson, B.E. and Frain-Bell, W. Plasma 8-methoxypsoralen concentrations in photochemotherapy of psoriasis, 47

Stolk, L., Siddiqui, A.H. and Cormane, R.H. Serum levels of trimethypsoralen after oral administration, 443

Stüttgen, G. *see* Marsch, W. Ch.

Sudan, B.J.J. and Sterboul, J. Nicotine: an hapten (Correspondence), 349

Summerly, R. *see* Messenger, A.G.

Szabó, E., Husz, S. and Kovács, L. Coexistent atypical bullous pemphigoid and systemic lupus erythematosus, 71

Taaning, E. *see* Worm, A.-M.
Tabiowo, A. *see* King, I.A.
Takahashi, K. *see* Ishii, M.
Tanaka, A. *see* Ishii, M.
Taniguchi, S. *see* Miyachi, Y.
Taylor, R.R. *see* Rhodes, E.L.
Thambiah, A.S. *see* Premalatha, S.
Thone, A.W. Indonesian boy with lichen urticatus, 98
Thöne, A.W. *see* Folkers, E.
Thorsby, E. *see* Falk, E.S.
Thyresson, N. Report on the meeting of the international committee of dermatology, 227
Tongio, M.M. *see* Eberst, E.
Topi, G.C., D'alessandro, G.L., Cancarini, G.C., De Costanza, F., Griso, D. and Ravelli, M. Porphyria cutanea tarda in a haemodialized patient, 579
Trotter, M.D. *see* Davies, H.W.
Tsambaos, D. and Orfanos, C.E. Ultrastructural evidence suggesting an immunomodulatory activity of oral retinoid. Its effect on dermal components in psoriasis, 37
Tucci, A. *see* Finzi, A.F.
Turbitt, M.L. and Mackie, R.M. Loss of β_2 microglobulin from the cell surface of cutaneous malignant and premalignant lesions, 507

Underwood, J.C.E. *see* Bleehen, S.S.

Valdimarsson, H. *see* Breathnach, S.M.
Van Baak, J. *see* Van der Horst, J.C.
Van de Kerkhof and Mali, J.W.H. Low-dose PUVA maintenance in psoriasis following Ingram therapy, 681
Van den Berg, W.H.H.W. Herpetiform pemphigus (Case report), 102
Van den Hurk, J.J.M.A. *see* Gommans, J.M.
Van der Horst, J.C. and Dirksen, H.K. Zosteriform lentiginous naevus (Case report), 104
Van der Horst, J.C. and Van Baak, J. Pigmented naevi following Stevens-Johnson syndrome (Case report), 106
Van der Horst, J.C. *see* Neering, H.
Van der Loo, E.M., Van Vloten, W.A., Cornelisse, C.J., Scheffer, E. and Meijer, C.J.L.M. The relevance of morphometry in the differential diagnosis of cutaneous T cell lymphomas, 257
Van der Loo, E.M. *see* Willemze, R.
Van Erp, P.E.J. *see* Roelfzema, H.
Van Ketel, W.G. and Kernekamp, A.S.-V.W. Contact allergy to clioquinol and neomycin (Summary), 100
Van Ketel, W.G. *see* Boonk, W.J.
Van Ketel, W.G. *see* Bruynzeel, D.P.
Van Ketel, W.G. *see* De Haan, P.
Van Vinninghe, A.-L. *see* Korthals, H.R.
Van Vloten, W.A. *see* Van der Loo, E.M.
Van Vloten, W.A. *see* Willemze, R.
Verbeeten, B. *see* De Groot, W.P.
Vermorken, A.J.M. *see* Wetterings, P.J.J.M.
Vreeswijk, J. Extension of the molluscum contagiosum lesion (Title), 101

Warin, R.P. Factitious urticaria: red dermographism, 285
Watanabe, S. *see* Horio, T.
Webster, G.F., McGinley, K.J. and Leyden, J.J. Inhibition of lipase production in *Propionibacterium acnes* by sub-minimal-inhibitory concentrations of tetracycline and erythromycin, 453
Weissbluth, M., Esterly, N.B. and Caro, W.A. Report of an infant with GM_1 gangliosidosis and extensive and unusual mongolian spots, 195
Wells, G.C. *see* Breathnach, S.M.
Wennersten, G. *see* Beitner, H.
Wetterings, P.J.J.M., Vermorken, A.J.M. and Bloemendal, H. A method for culturing human hair follicle cells, 1
Wigand, J.S. *see* Sklar, S.H.
Wilkinson, J.D. *see* Burge, S.M.
Willemze, R., Van Vloten, W.A., Van der Loo, E.M. and Meyer, C.J.L.M. Primary lymphoblastic non-Hodgkin's lymphoma of the skin, 333
Witkiewicz, I.M., Woerdeman, M.J. and De Groot, W.P. Tuberous sclerosis. Ultrastructure study of white spots in patients and parents (Summary), 100
Witkiewicz, I.M. *see* De Groot, W.P.
Woehler, M.E. *see* Morison, W.L.
Woerdeman, M.J. *see* Boorsma, D.M.
Woerdeman, M.J. *see* De Groot, W.P.
Woerdeman, M.J. *see* Witkiewicz, I.M.
Worm, A.-M., Taaning, E., Rossing, N., Parving, H.-H. and Clemmensen, O.J. Distribution and degradation of albumin in extensive skin disease, 389
Wranne, L. *see* Blom, I.
Wright, N.A. *see* Ralfs, I.
Wright, N.A. *see* Ralfs, I.G.
Wright, J.T. *see* Cowan, R.E.

Yadav, G. *see* Rehák, A.
Yesudian, P. *see* Premalatha, S.
Young, A.R. and Magnus, I.A. An action spectrum for 8-MOP induced sunburn cells in mammalian epidermis, 541

Zilkha, K.D. *see* Pembroke, A.C.
Zina, G. *see* Bernengo, M.G.

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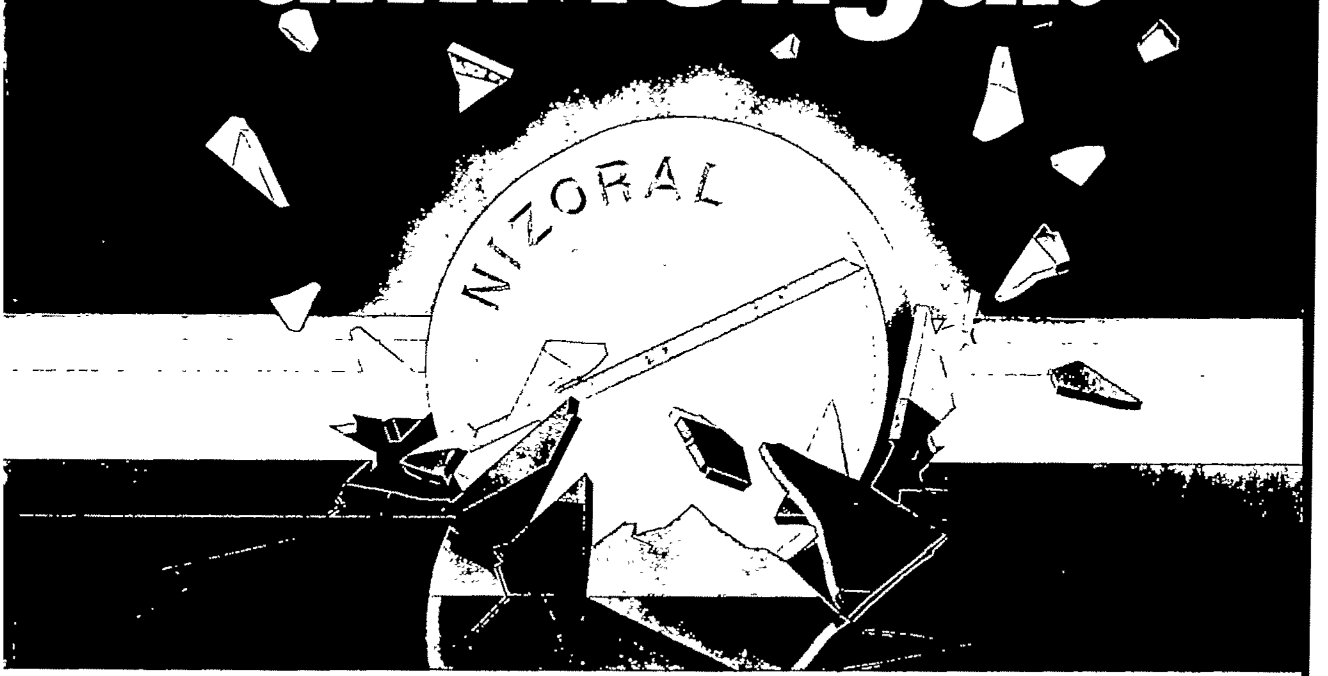

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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British Journal of Dermatology, Volume 104, Number 6, June 1981

CONTENTS

Clinical and Laboratory Investigations

PAGE

Dermatological non-disease: a common and potentially fatal disturbance of cutaneous body image 611
J.A.COTTERILL

Autoantibodies to an epidermal protease inhibitor in skin diseases 621
V.K.HOPSU-HAVU, KIRSTI KALIMO AND M.JÄRVINEN

Measurement of biological ageing in human epidermis 627
R.MARKS

Studies on the plasma membrane of normal and psoriatic keratinocytes. 3. Uptake of labelled sugars and their incorporation into glycoconjugates 635
H.ROELFZEMA, M.BERGERS, P.E.J. VAN ERP, J.M.GOMMANS AND P.D.MIER

Lectin binding studies on suspensions of isolated epidermal keratinocytes 641
J.M.GOMMANS AND J.J.M.A. VAN DEN HURK

Synthesis and turnover of membrane glycoconjugates in monolayer culture of pig and human epidermal cells 649
HELEN W.DAVIES AND M.D.TROTTER

Molecular basis of substrate and inhibitory specificity of tyrosinase: phenolic compounds 659
S.PASSI AND M.NAZZARO-PORO

Pharmacology and Treatment

Severe disorders of keratinization: effects of treatment with Tigason (etretinate) 667
RONALD MARKS, ANDREW Y.FINLAY AND PETER J.A.HOLT

The efficacy of an aromatic retinoid, Tigason (etretinate), in the treatment of Darier's disease 675
S.M.BURGE, J.D.WILKINSON, A.J.MILLER AND T.J.RYAN

Low-dose PUVA maintenance in psoriasis following Ingram therapy 681
P.C.M.VAN DE KERKHOF AND J.W.H.MALI

Effect of topical 17α propyltestosterone on sebum excretion in man 685
FERGUS LYONS AND SAM SHUSTER

Case Reports

Sclerosing lymphangitis of the penis: a lymphangiofibrosis thrombotica occlusiva 687
WOLFGANG CH.MARSCH AND GÜNTER STÜTTGEN

Allergic contact dermatitis due to an epoxy acrylate 697
JAMES R.NETHERCOTT

Cold urticaria with persistant weals 705
LENNART JUHLIN

Brief Communications

An unusual adverse reaction to Ro 10-9359 707
K.MOULOPOULOU-KARAKITSOU

UV-induced pyrimidine dimers and rosacea 711
ENRICO NUNZI, ALFREDO REBORA, JAN J.CORNELIS AND RUDY H.CORMANE

Comment Article

Mixed connective tissue disease 713
C.BLACK

Correspondence 721

Book Reviews 723

Editorial 729

News and Notices 730

Index 733